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Datum: 14.12.2009

Success of autogenous tooth transplantation: a retrospective and prospective approach

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Eindverhandeling voorgedragen tot het bekomen van de graad master in de biomedische wetenschappen klinische moleculaire wetenschappen

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Abbreviations

OMF: oral maxillofacial surgery
PDL: periodontal ligament
CBCT: cone-beam computed tomography
CT: computed tomography
3D: three-dimensional
2D: two-dimensional
STL: stereolithography
LDF: laser Doppler flowmetry
GI: gingival index
sRGB: standardized red-green-blue
CIE: International Commission on Illumination
SD: standard deviation
OPG: orthopantomograph
OD: odds ratio
BIOMED: Institute of Biomedical Research
DICOM: Digital Imaging and Communication in Medicine

Preface

This thesis project is the result of a 8-month internship at the department oral maxillofacial surgery (OMF) of the St. John's Hospital in Genk. A period in which I acquired new knowledge, made new friends and have learned al lot about myself. Working on my thesis has been exciting, interesting and sometimes frustrating. I would like to say a special thanks to all the people who have encouraged and assisted me.

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Finally, I would like to say thank you to my parents for the continuous support they give me in everything I do. Thank you for giving me the opportunity to study and to complete this master. I would also like to thank my boyfriend, my sister and all my friends for just being there for me and listening when I needed someone to talk to, to stress out or just to be happy.

Hanne

Abstract

Introduction Autogenous tooth transplantation can be defined as the surgical movement of a tooth from one position in the mouth to another. A prerequisite for the use of autotransplantation as a treatment option is a thorough knowledge of the expected long-term success rate. Literature contains only a few follow-up studies about long-term results of transplanted canines. For this reason, the first aim was to perform a retrospective study in order to determine the long-term survival and success rates of autogenously transplanted canines. In addition, the influence of various parameters such as age, gender, root formation and others on the long-term success rate was investigated. Based on the available literature, the most important factor that influences the success rate of transplantation is the preservation of the periodontal ligament attached to the donor tooth. Therefore, the second aim is to perform a prospective study in order to optimize and describe the protocol for the production of a tooth transplantation template. More specifically, different methods of image segmentation will be investigated. Once the most appropriate segmentation method has been determined, a case study was performed.

Material and methods Patients who had canines transplanted from 1995 until 2002 were contacted. From the patients who were willing to participate, all pre- and peroperative parameters which could influence the outcome of transplantation were looked up in the patient files. Each transplanted canine was clinically and radiologically evaluated. Logistic regression analyses were performed to determine the influence of various parameters on the success rate.

The material of the prospective study consisted of six dry human skulls which were scanned with the cone-beam device Galileos. After scanning the skulls, ten randomly chosen molar teeth were extracted and scanned again. The three-dimensional images of the tooth in the maxilla and the extracted tooth were segmented with the same method. Three segmentation methods were compared with respect to surface distance and segmentation time. The template used in the case study was printed out by the stereolithography technique.

Results Sixty patients, in whom 74 teeth were transplanted, volunteered to participate in the retrospective study. The mean follow-up period was 11 years. The survival rate was 75.7% because 18 transplanted teeth were lost before examination. Based on the clinical and radiological examination, the success rate for all transplanted teeth was 58.1% because 43 transplanted teeth were evaluated as clinical successful. The significant parameter in determining the success rate of autotransplantation was age at transplantation.

It was not possible to discriminate teeth from the alveolar bone by doing thresholding, therefore this segmentation method was excluded from the study. No significant difference in surface distance was found between region- and edge-based segmentation. However, the time needed to complete region-based segmentation was significantly lower than the segmentation time for the edge-based method.

Conclusion Autotransplantation of impacted canines may have a successful outcome 11 years after transplantation. The success rate increases when performing the transplantation at a younger age. In the future, the success of tooth transplantation may be further increased by using a tooth transplantation template.

Introduction

1. Dental anatomy

A study of transplantation of teeth requires first learning about the morphology of the teeth. The dental anatomy is already extensively described in several books [1, 2], therefore only a short description will be given here. Humans have two sets of teeth, 20 primary teeth which will be eventually replaced by 32 permanent teeth. The teeth are equally divided between the upper (maxilla) and lower jaw (mandible). There are four different types of teeth: incisors, canines, premolars and molars. Each tooth consists of a crown and a root (Figure 1 *left*). The crown is visible in the mouth and covered with enamel, while the root is buried in the alveolar bone and covered with cementum. The tooth is mainly composed of dentin, a calcified tissue surrounding the pulp cavity of a tooth. The pulp cavity consists of pulp tissue and can be divided into two compartments, the pulp chamber in the crown portion and the pulp canal in the root portion. Anterior teeth (incisors and canines) usually have one root canal, whereas posterior teeth (premolars and molars) have multiple canals. The pulp tissue contains blood vessels, nerves and cellular connective tissue. The nerves and blood vessels enter and leave the tooth through the apical foramen, an opening at the apex of the root [1, 2].
The crowns of the teeth have different surfaces which are named according to their positions (Figure 1 *right*). In the incisors and canines, the surfaces facing the lips are called *labial* surfaces. The surfaces toward the cheek in premolars and molars are called *buccal* surfaces. Labial and buccal surfaces are collectively termed *facial* surfaces. All surfaces related to the tongue are called *lingual* surfaces. Furthermore, each tooth has two proximal surfaces, one that is orientated toward the midline of the dental arch (*mesial* surface) and another faced away from the midline (*distal* surface). Finally, the cutting edge of an anterior tooth is called *incisal* surface while the chewing plane of a posterior tooth is termed *occlusal* surface [1, 2].

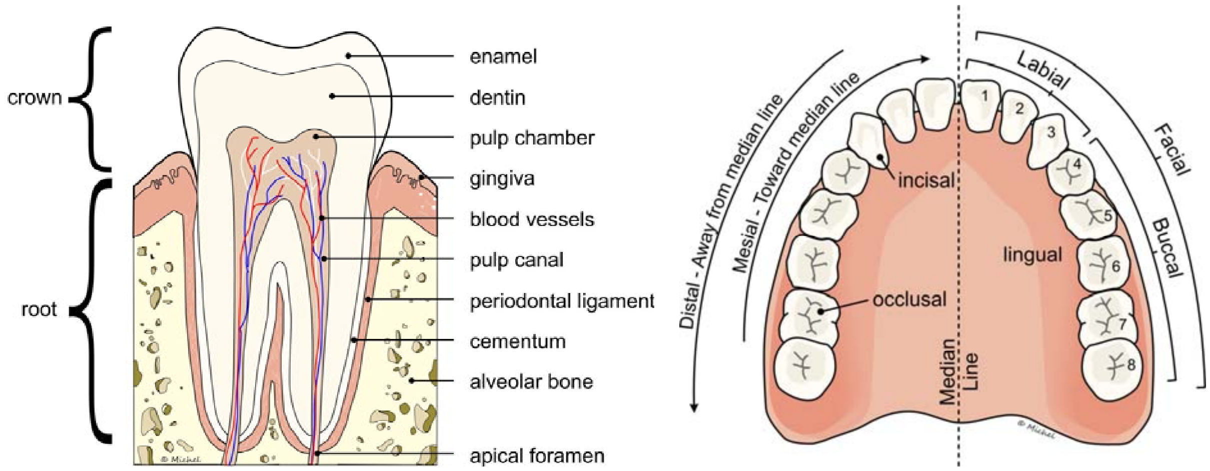

Figure 1: A schematic picture of a normal tooth is shown at the *left*. A schematic picture of the tooth surfaces, named according to their positions, is shown at the *right*.

2. Transplantation of teeth

Transplantation of teeth can be defined as the surgical movement of a tooth from one position in the mouth to another. As with the transplantation of other tissues, the transplantation of teeth can be categorized into autogenous (donor and recipient are the same individual) and allogenous (donor and recipient are not the same individual) tooth transplantation. The earliest reports of tooth transplantation involve slaves in ancient Egypt who were obligated to give their teeth to the pharaohs. Allotransplantation was eventually abandoned because of a low success rate due to histoincompatibility and disease transmission. However, autotransplantation is a viable treatment option for tooth replacement when patients are carefully selected [3, 4].

Although there are many reasons for autotransplantation of teeth, the most common indication is tooth loss. Teeth can be congenitally missing or lost due to incurable trauma, dental caries or periodontal diseases. Most frequently, a third molar is transferred to the site of an unrestorable first molar. Moreover, autotransplantation is also applied for repositioning of impacted teeth to their normal position (transalveolar transplantation). A tooth is called impacted when it fails to erupt properly due to obstruction by another tooth, bone or soft tissue [5]. Autotransplantation of impacted canines is considered as an alternative when surgical exposure and orthodontic realignment are not feasible. Impacted third molars are transplanted to substitute for first or second molars. In the following paragraphs the criteria for success and the causes of failure after autotransplantation will be considered.

2.1 Criteria for success after autotransplantation

The factors that influence the survival and success rates of autotransplantation have been extensively investigated. Successful transplantation depends on specific requirements of the patient, the donor tooth, the recipient site and the surgical technique.

2.1.1 Candidate criteria

Patient's selection has to be done carefully. First of all, candidate patients are required to be in good health. They must agree with the chosen treatment option and be cooperative and comprehensive because they will need to return for several follow-up visits. Furthermore, they should be able to follow post-operative instructions such as take soft diet and maintain their oral hygiene. Regular dental care plays an important role in achieving successful results. In addition, autotransplantation is preferably done in younger patients (under 20 years of age) this can be explained by the stage of root development (see: paragraph 2.1.2). Most importantly, the candidates must possess a suitable donor tooth and recipient site [4, 6, 7].

2.1.2 Donor tooth criteria

Success of autotransplantation of teeth depends mainly on the vitality of the periodontal ligament (PDL) attached to the donor tooth. If a tooth has a healthy and undamaged PDL, the success rate after transplantation is optimal. The PDL is a group of specialized connective tissue fibres that connects the tooth to the alveolar bone. Lamina dura is a radiographic term denoting the alveolar bone that lies adjacent to the PDL. Because cells of the PDL can be damaged mechanically during extraction, the donor tooth should be positioned at the donor site in such a way that extraction can be performed as atraumatic as possible.

Generally, premolar transplantations show the best prognosis while the prognosis of canine transplantations is the worst. This may be due to variations in morphology and surgical access. When the accessibility is less, the chance to damage the tooth during extraction is higher [6, 8]. The success of autotransplantation increases when single-rooted teeth are transplanted. Teeth with multiple roots or curved roots can make tooth removal difficult, in these cases the success rate diminishes. Besides root shape, the stage of root development at the time of transplantation is also an influencing factor for success. The more developed the root, the greater the possibility that the PDL will be injured during extraction. Little force is needed to remove immature teeth, while fully erupted teeth are strongly attached to the alveolar bone. Therefore, the success rate of autotransplantation of mature teeth is less than that for developing teeth. Transplantation of teeth at the stage between ½ - ¾ of its final root length shows the best prognosis (Figure 2). If a non-functioning tooth, such as an impacted tooth, is chosen as a donor tooth, the success rate decreases because the PDL may be inadequate to develop a functioning attachment in its new position [3, 6-9].

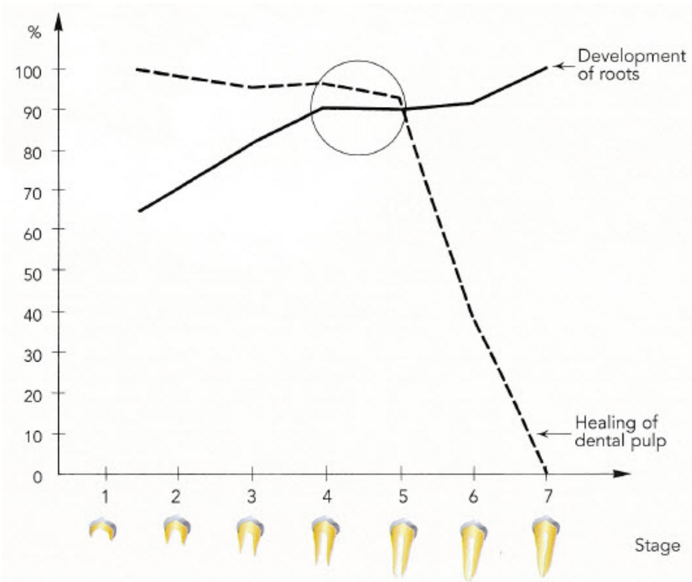

Figure 2: Donor teeth at the development stage 3 or 4 (meaning between ½ - ¾ of its final root length) are ideal for transplantation [7].

Both teeth with open and closed apices can be chosen as donors. When the donor tooth has an open apex the chance of revascularization increases. Revascularization has two different meanings depending on the condition of the pulp tissue. When pulp tissue of the donor tooth has become necrotic after transplantation, revascularisation refers to ingrowth of highly vascularised connective tissue into the pulp space. Afterwards, this connective tissue will be replaced by tissue similar to bone and cementum, resulting in obliteration of the pulp. On the other hand, the original pulp tissue of the transplanted tooth can sometimes survive transplantation. In this case revascularization refers to the process in which blood vessels already present in the pulp of the transplanted tooth anastomose with blood vessels from the PDL. In teeth with open apices, successful transplantation without the need for further endodontic therapy (see: paragraph 2.2) is usually seen. However, if any sign of pulp infection is detected, endodontic treatment should be initiated as soon as possible. Although revitalization of teeth with closed apices has also been reported, in most cases endodontic treatment is needed to obtain successful transplantation [10]. Damage to the epithelial rests of Malassez during extraction is also a criterion that could influence the outcome of transplantation. The epithelial rests of Malassez form a specific cell population in the developing and established PDL. A reduction of the epithelial rests of Malassez can lead to the development of ankylosis [11, 12].

2.1.3 Recipient site criteria

The remaining tooth in the recipient site should be extracted very carefully to retain sufficient alveolar bone support. Adequacy of bone support in all dimensions is essential to stabilize the transplanted tooth. In addition, the presence of PDL in the recipient socket has a positive influence on the wound healing after transplantation. The prognosis improves when teeth are transplanted into a recipient site with remaining PDL. Furthermore, the recipient site should be free from acute infection and chronic inflammation. Another important criterion is the adaptation between the donor tooth and recipient site. The recipient socket should be slightly larger than the donor tooth. In this case, repair of the root surface attachment can take place before bone tissue reaches the root. When the recipient socket is too large relative to the size of the donor tooth, bacterial invasion may occur [4, 7].

2.1.4 Surgical procedure

The outcome after autotransplantation of teeth depends on the experience of the surgeon. The surgeon must develop skills and expertise to extract the donor tooth and place it into the recipient site without damaging the PDL. Because of the correlation between extra-oral time and damage to the PDL, the donor tooth must be transplanted as quickly as possible. The extra-oral time can be defined as the period from the extraction of the donor tooth until its insertion into the recipient site [13]. Although there is no critical margin for the extra-oral time, it is obvious that a short extra-oral time is favourable for the survival of the PDL [14]. Furthermore, the transplanted tooth may not have any traumatic contact with its antagonist at the occlusal plane. A traumatic occlusion leads definitely to failure of the transplantation. Another factor that affects the healing of a transplanted tooth is the fixation method and duration after transplantation. Splinting does not improve periodontal healing after transplantation, but results in an increase in the amount of replacement resorption. However, stabilization of transplanted teeth is necessary until attachment is adequate. Non-rigid splinting provides physiologic movement of the tooth, thus minimizing the risk of resorption [3, 6, 7, 9].

2.2 Causes of failure after autotransplantation

The most common cause of failure following autotransplantation is chronic root resorption. When a donor tooth with partial or total lack of vital PDL is transplanted, root resorption occurs. The amount of damaged PDL is an important factor in determining which type of root resorption will occur. When a tooth with extensive loss of vital PDL is transplanted replacement resorption occurs (Figure 3a). **Replacement resorption** or ankylosis is an irreversible process in which the root is resorbed and replaced by bone. The speed of this remodelling depends on the age of the patient.

In adult patients the teeth are lost very slowly and can maintain function and aesthetics for many years. In contrast, teeth are lost quickly and intervention is necessary in young children. Clinically, ankylosis is found between four weeks to one year after transplantation. **Inflammatory resorption** is observed when the pulp becomes infected, the bacteria in the pulp canal act as a constant stimulus for inflammation (Figure 3b). This type of resorption can be arrested if discovered in its early stage and endodontic treated. During endodontic treatment, which is also known as root canal therapy, the root-canal system of the teeth becomes disinfected preventing bacteria to penetrate into the surrounding tissues. Inflammatory resorption can be diagnosed within two months after transplantation. The last type of root resorption is called surface resorption and is limited to the surface of the cementum or dentin (Figure 3c). **Surface resorption** is the result of limited partial injury of the PDL and, in most cases, it undergoes spontaneous repair [6, 7, 15, 16].

Another cause of failure after transplantation is periodontitis. Periodontitis can be defined as inflammation of the supporting structures of the tooth. These structures include alveolar bone, cementum, gingiva and PDL, commonly referred to as the periodontium. Periodontitis involves progressive loss of the alveolar bone around the tooth, and if left untreated, can lead to loosening and subsequent loss of the tooth [6].

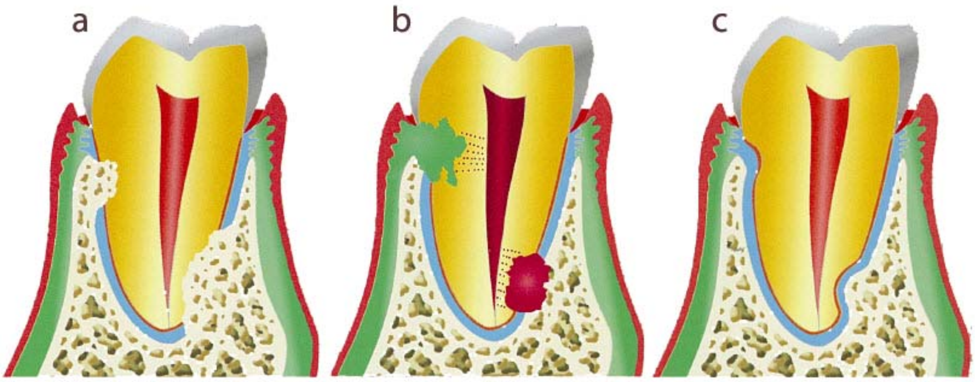

Figure 3: Classification of root resorption. A) Replacement resorption. The root is resorbed and replaced by bone. B) Inflammatory resorption. The infected pulp tissue acts as a constant stimulus for inflammation. C) Surface resorption. This type of resorption is limited to the surface of the cementum or dentin [7].

3. Impaction of canines

The maxillary canine is the most frequently ectopic tooth in the anterior part of the mouth. The **incidence** of impacted maxillary canines varies from less than 1% to 3% [17]. Maxillary impacted canines are more often located palatal (85%) than labial (15%). Furthermore, impaction of canines is more common in female patients than in male patients and is often only discovered in the late teens. In all patients with ectopic canines, 8% concerns bilateral displacement. The mandibular canine is much less of concern because it is ten times less frequently impacted. Mandibular impacted canines can be located lingual or labial. Impacted canines cause great problems in treatment planning and are a specific reason for referral to both the orthodontist and the oral surgeon. Patients are impaired functionally and aesthetically when the canines do not erupt [4, 6, 18, 19].

The exact **etiology** of impacted canines remains unknown. However, it is clear that the cause of labial displacement is different from that involved with palatal displacement of the canine. Maxillary canines have the longest period of development and the longest eruption path. During development, their crowns contact lateral incisor roots. Therefore, missing lateral incisors have been suggested to contribute to the etiology of palatal impacted canines. The lack of proper guidance by the lateral incisor root results in a more palatal movement of the canine. On the other hand, canines may become labial impacted as a result of arch length discrepancy and space deficiency. Because the teeth immediately adjacent to the canine erupt before the canine, the close proximity of these adjacent teeth will prevent the canine from moving into the arch. Other possible causes of impacted canines are genetic predisposition, supernumerary teeth and ankylosis [5].

The permanent maxillary canine normally erupts at the age of 11 to 13 years. Because unerupted permanent maxillary canines cause the patient relatively few problems, most patients are frequently unaware of the presence of an impacted canine. The discovery of canine impaction is therefore usually made at the time of routine dental examination. **Diagnosis** of impacted teeth depends on clinical inspection and radiological investigation. It is important not only to locate the unseen tooth, but also look for possible complications of the untreated impacted tooth. The most common complication is resorption of the root of the adjacent lateral or central incisor. Due to superimposition on plain film radiographs (periapical and panoramic radiographs), it is not always possible to define the presence of incisor root resorption. To provide the maximum positional information and reduce the dosage of ionizing radiation to the minimum, cone-beam computed tomography (CBCT) should be used [5].

After a clinical and radiographic evaluation, the appropriate **treatment** can be defined. If the canine is in good position and there is no evidence of root resorption of the adjacent teeth, no treatment except periodically monitoring is recommended. Another treatment option is extraction of the primary canine to facilitate eruption of the impacted canine. However, the traditional treatment for ectopically positioned canines is surgical exposure and orthodontic realignment. When the position of the canine is not such that orthodontic treatment would be feasible, autotransplantation of teeth can be considered. Finally, if the degree of displacement of the canine does not allow for surgical reposition or transplantation, extraction of the impacted canine is indicated [4, 6, 17-19].

4. Computer-aided rapid prototyping

Rapid prototyping can be defined as a group of technologies used to construct physical prototypes layer-by-layer according to computer-aided design data sources [20]. Computer-aided rapid prototyping can be used for the fabrication of a donor tooth model. Lee et al. (2001) already demonstrated a lower extra-oral time and less injury to transplanted teeth after performing autotransplantation using computer prototyping to obtain a duplicate form of the donor tooth [13, 21]. The general process of rapid prototyping consists of **three major steps** (Figure 4).

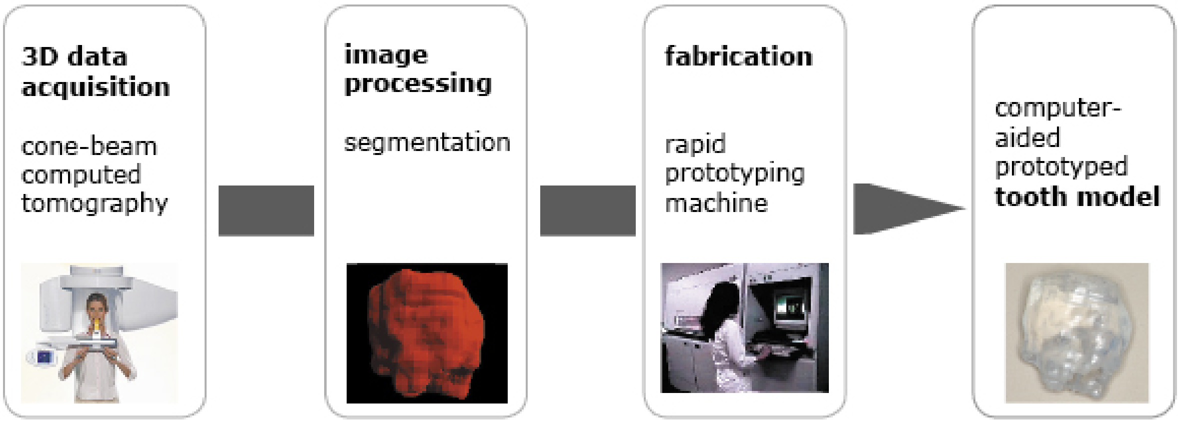

Figure 4: The general process of rapid prototyping tooth model construction consists of three major steps: three-dimensional data acquisition, image processing and prototype fabrication.

First, computed tomography (CT) is used to obtain three-dimensional (3D) data of teeth. Recently, digital volume tomographic machines which use the cone-beam principle have become available. The cone-beam system consists of an X-ray source and a two-dimensional (2D) detector which perform a single 360° rotation around the patient's head. The raw data are reconstructed directly into axial, panoramic and transaxial views. The greatest advantage of CBCT compared with conventional CT is that it acquires the maximum positional information in one rotation at reasonable low levels of radiation dosage [5].

Secondly, image segmentation is required to extract the region of interest from surrounding tissues. Image segmentation refers to the process of partitioning a digital image into multiple regions in order to make the representation of the image easier to analyse [22]. More precisely, to segment a 3D image means to assign a label to every voxel in the image such that voxels with the same label share a certain property such as color, intensity or texture. For this purpose, reconstruction algorithms included in software programs are used [13, 21, 23].

Thirdly, 3D digital data are exported to a rapid prototyping machine for fabrication. A large number of fabrication technologies are available; their main differences are found in the way layers are built to create parts. Stereolithography (STL) is the most widely used rapid prototyping technology. STL builds physical models a layer at a time by tracing a laser beam on the surface of a vat of liquid photopolymer. Three-dimensional printing is a unique form of printing which is related to rapid prototyping technology. A 3D object is created by layering and connecting cross sections of material. Three-dimensional printers are generally faster, more affordable and easier to use than other fabrication technologies [21].

Research aims

The main problem with autotransplantation of impacted canines is their accessibility. Due to the difficult position of impacted canines there is a higher risk of damage to the root surface during extraction. Because impacted canines are a particular challenge in children and adolescents, the transplanted tooth should preferably adapt to growth and developmental changes in the oral region. Furthermore, the tooth should have the potential for long-term, even lifelong survival. Literature contains only a few follow-up studies about long-term results of transplanted canines. For this reason, the **first aim** was to perform a **retrospective study** in order to determine the long-term survival and success rate of autogenously transplanted canines. In addition, the influence of various parameters such as age, gender, root formation and others on the long-term success rate was investigated.

Based on the available literature, the most important factor that influences the success rate of transplantation is the preservation of the periodontal ligament attached to the donor tooth. Therefore, the **second aim** is to perform a **prospective study** in order to optimize and describe the protocol for the production of a tooth transplantation template. More specifically, different methods of image segmentation will be investigated. Once the most appropriate segmentation method has been determined, a case study will be performed. The designed transplantation template can be used in the surgical procedure to prepare the recipient socket instead of the donor tooth itself.

The outcome of both the retrospective and prospective study will make it possible to refine the clinical transplantation procedure in order to make autogenous tooth transplantation more predictable and obtain even better results in the future.

Retrospective study

1. Material and methods

1.1 Patients

A total of 133 patients had undergone canine transplantation (164 canines) from 1995 until 2002. First, considerable efforts (letter, phone call…) were made to contact these patients because of the long-term period since their operations. From the patients who were willing to participate in this study, baseline information was looked up in their files. All parameters which could influence the outcome of transplantation were recorded (Table 1). These parameters have been written down at the time of transplantation. Moorrees' classification (see: Introduction, Figure 2) for the developmental stages of roots was used as a basis to estimate the stage of root development of each transplanted tooth at the time of transplantation. For this purpose, previous analog radiographs were observed. It was not always possible to document the condition of the root and apex of impacted canines.

Table 1: Pre- and per-operative parameters which could have an influence on the overall success rate of transplantation.

Parameters
Patient: - **Gender** - **Age** at the time of transplantation
Transplantation area: - **Position** of the canine - Previous **surgical exposure** - **Sufficient space** for transplantation
Transplanted canine: - **Root development** at the time of transplantation - **Apical anomaly** (curved apex) - **Unilateral / bilateral** transplantation
Adjacent teeth: - **Root resorption**
Per-operative: - **Damage** of the PDL - **Fixation** by orthodontic arch/trauma splint - **Occlusion**

1.2 Surgical procedure

All transplantations were performed by the same experienced oral surgeon, according to a strict protocol. Before a surgical approach was made, a radiographic examination had already revealed the location of the impacted canine. Furthermore, the graft and the recipient site dimensions were checked to be compatible. The surgical procedure differed according to the site of impaction but a general principle is described below.

First, the surgical sites were disinfected and a local anaesthetic was injected. The remaining primary canine, if it was still present, was extracted and a trapezoidal incision was made which ensured intact mesial, distal and palatal gingiva at the graft site. To prepare the recipient socket,

osteotomy was performed using a surgical bur and chisels. The tooth roots that were mesial and distal to the osteotomy site must remain intact. If the recipient site was too small for the donor tooth, the socket area was expanded by loosening the vestibular and palatal bone plates with chisels. The socket for the graft should be slightly larger than the graft. Next, the crown of the impacted canine was exposed and the tooth was removed with a periosteal elevator. The donor tooth was extracted slowly and as atraumatically as possible. It is important that the instrument contacts only the crown and not the root surface in order not to damage the PDL. Next, the donor tooth was placed into the recipient socket without any pressure. Obstacles in the socket wall were removed as encountered. The trapezoidal flap was repositioned and sutures were placed. To optimize reattachment and block bacterial invasion, the gingival flap must close tight around the donor tooth. Finally, the donor tooth was splinted with a suture splint or with an acid-etch composite and orthodontic wire. The occlusion was checked to ensure that the transplanted tooth had no traumatic contact with its antagonists at the occlusal plane (infra-occlusive position).
In Supplemental section 1 the surgical procedure is shown for a maxillary canine in labial deviation. The surgical removal of an ectopic palatal located canine was more difficult compared with canines in a labial position due to the close proximity between the crown of the canine and the roots of the central or lateral incisor [6, 7, 16].

1.3 Clinical and radiological examination

Before the examination started, each patient signed a written informed consent statement approved by the St. John's Hospital Ethics Committee (see: Supplemental section 2). Each transplanted tooth was clinically and radiologically assessed by the same examiner to determine the success rate. Clinically successful transplantation was achieved when the transplanted tooth was still present in the mouth without any patient complaints but with possible transient root resorption or endodontic treatment. When the transplanted canine showed progressive root resorption, pathological pockets, extensive loss of alveolar bone or an apical inflammation, the transplantation was defined as clinically unsuccessful. The **success rate** was calculated as the percentage of clinically successful transplantations relative to the total number of transplantations in the sample. A distinction was made between the success rate and the survival rate. The **survival rate** was defined as the percentage of transplanted teeth still present at the examination relative to the total number of teeth that were transplanted.

1.3.1 Mobility assessed by the periotest

The mobility of the transplanted tooth was tested by means of the periotest (Medizintechnik Gulden, Modautal, Germany) [24]. The periotest is a non-invasive, electronic device which measures the reaction of the PDL on a defined force applied to the tooth crown. The normal periotest values for women range between 00 and 06. For men, the normal periotest values range between -01 and 04. A periotest value which is lower than the normal periotest values means that the tooth shows less mobility, pointing towards ankylosis. When the periotest value is higher than the normal ranges, the transplanted tooth is clinical mobile. Periotest measurements were taken in accordance with manufacturers' instructions. First, a function check was done to be sure that the periotest worked properly. Next, the patient's head was placed in the headrest with the transplanted tooth perpendicular to the floor. The handpiece was held as horizontally as possible and at a distance of 0.7 to 2.0mm from the tooth. The tooth was out of occlusion and percussed at the middle of the crown. The measurements were all taken twice by one examiner.

1.3.2 Pulptesting to determine tooth vitality

Tooth vitality of the transplanted canines was only examined if no endodontic treatment was performed. Thermal and electrical pulp tests were applied to determine the innervation of the teeth. A cold-test (Fricar Odontotest) based on carbon dioxide snow was performed. First, the teeth were dried and subsequently, the produced carbon dioxide stick was applied on the teeth. Because of its low temperature (-78°C) carbon dioxide snow gives very consistent and reliable results. In addition, the vitality of dental pulp was examined by using electric stimulation. During the electrical pulp test, the current stimulates intradental nerves and causes acceptable pain to the patient. The reactions to the cold test and the electrical test were quantified with "yes" or "no" answers by the patients. If the nerves could be stimulated, the pulp innervation was assumed to be intact.
Furthermore, laser Doppler flowmetry (LDF) was used to assess pulp vascularisation. LDF is a unique method for continuous monitoring of tissue blood perfusion. In theory, nonvital teeth should provide very low pulpal blood flow values because there is no pulp blood circulation. In contrast, healthy teeth with a good blood flow should show a clear pulsatile wave. LDF evaluation was performed using a DRT 4 LDF monitor (Moor Instruments Ltd, Axminster, Devon, UK) with a laser diode operating at a wavelength of 780 to 820nm and a maximum accessible power of 1.6mW. The DRT 4 recorded tooth signals at a bandwidth of 3.1 kHz with a time output constant of 0.1s. Each probe was labelled and calibrated following manufacturer's instructions. The standard probe location was selected 2 to 3mm coronal to the level of the gingiva because adjacent tissue could lead to contamination of the signal. The probe tip was held firmly while it was in contact with the tooth. A recording was made on each transplanted tooth for at least a minute to obtain an uninterrupted trace. All teeth were examined by the same observer under standardised environmental conditions. The data were directly acquired from the DRT 4 onto a computer and analysed using the compatible software program (moorSOFT/ DRT4, Moor Instruments Ltd, 2004).

1.3.3 Probing the pocket depth and scoring the gingival inflammation

A pocket can be defined as the area of potential space between a tooth and the surrounding gingival tissue [1]. To measure the pocket depth, a calibrated periodontal probe (Hu-Friedly, Leimen, Germany) was inserted with light pressure into the gingival pocket at four defined locations (facial, mesial, distal, and lingual). The probe was kept parallel to the root of the tooth and the first marking visible above the pocket indicated the measurement of the pocket depth. Pockets with a depth of more than 3mm were rated as pathological. Furthermore, the gingival inflammation was scored according to the Gingival Index (GI) by Loe and Silness (Table 2) [25].

Table 2: The Gingival Index (GI) by Loe and Silness [25].

Appearance	Bleeding	Inflammation	GI
normal	no bleeding	none	0
slight change in color and mild edema with slight change in texture	no bleeding	mild	1
redness, hypertrophy, edema and glazing	bleeding on probing/pressure	moderate	2
marked redness, hypertrophy, edema, ulceration	spontaneous bleeding	severe	3

1.3.4 Colour

In collaboration with Doctor Van Poucke (anaesthesiology, St. John's Hospital, Genk) the transplanted canine colour was compared with the colour of the non-transplanted canine in the opposite jaw. Clinical intra-oral digital images were taken with a Nikon 3D digital camera (Nikon Corp. Tokyo, Japan). In order to remove most of the variability introduced by changes in lightning, exposure and white balance, the acquired images were calibrated. The calibration involves transforming the pixel values coming from the camera to pixel values defined in the standardized red-green-blue (sRGB) colour space. This colour space is based on the fact that all colours can be reconstructed by a suitable combination of three base colours: red, green and blue. A prerequisite for this calibration is that the acquired image must include a small colour card close to the region of interest (Figure 5). This colour card contains a number of colour squares with known colorimetric properties. When the colours in those squares are transformed correctly to their known sRGB values, a suitable mathematical transformation can be determined that is valid for all the pixels in the image [26, 27].

Figure 5: Image of the colour card which was used for the calibration of two images

The perceptual colour differences between the transplanted tooth and the non-transplanted tooth were expressed in CIE units. The International Commission on Illumination (CIE) calls their color difference metric deltaE*ab, which is proportional to the colour difference as seen by the human observer. Theoretically 1 unit is the 'just noticeable colour difference' and anything above 5 units is clearly noticeable [28, 29]. The difference was calculated from five different locations on the transplanted tooth. From these five deltaE*ab values the average and standard deviation (SD) was calculated. When the average deltaE*ab value was above 5, it could be concluded that there was a significant colour difference between the transplanted canine and the non-transplanted canine.

1.3.5 Intra- and extra-oral radiography

During the radiological examination, periapical films (intra-oral) and panoramic radiographs (extra-oral) of all transplanted teeth were taken. Periapical radiographs highlighted the teeth from either the upper or lower jaw in one part of the mouth. It was important to image the total length of the teeth with at least 2mm of the periapical bone. A film was placed into the mouth of the patient using a plastic film holder device in the required position to obtain a good-quality image. Once the film was positioned and stable, the cone of the X-ray unit was directed toward the film causing the film to be exposed. The exposure parameters for periapical radiographs were 70 kV, 7mA and 0,06s. Panoramic radiographs, more specifically orthopantomographs (OPG), were made with the film outside the mouth. The panoramic view has the advantage of simply and quickly offering a good scan of all teeth on both upper and lower jaws. The exposure parameters for panoramic radiographs were 66kV, 10mA and 5s.

In addition, CBCT images were collected from all patients to evaluate the radiological parameters. At the department OMF of the St. John's Hospital in Genk, the cone-beam scanner Galileos (Sirona, Bensheim, Germany) is used. The scan parameters for a 3D volumetric cone-beam scan from Galileos were 85kV, 7mA and 14s. The Galileos device features a fixed field of view of 15cm resulting in a scan volume of 15cm x 15cm x 15cm with a resolution of 300µm.

Radiological examination contributed to the evaluation of root resorption (intern/extern), pulp obliteration, lamina dura formation, alveolar bone loss and apical inflammation. In contrary to preoperative parameters (root development and apical anomaly) which had to be estimated from analog radiographs, postoperative parameters could be evaluated from digital radiographs.

1.4 Statistic analysis

Logistic regression analyses were performed to determine the influence of the different parameters on the success rate of autotransplantation of canines. **Univariate** logistic regression analyses were carried out to identify the effect of each parameter separately on the success rate. Parameters with $p<0.25$ on univariate logistic regression or those thought to be important on logical and medical grounds were entered into a multivariate logistic regression model. **Multivariate** logistic regression models were fitted in order to analyse the relative involvement of the various parameters in the success rate of autotransplantation. To compare the models in their goodness of fit, Akaike and Bayesian information criteria were used. Fitting the models was performed on complete observations. Mixed models were used to take into account correlation because some patients had more than one tooth transplanted.

In order to estimate the inter-examiner variation all radiographs were evaluated by a second examiner. **Simple κ** statistics were computed to determine the inter-examiner agreement. The **Kaplan-Meier** method was used to estimate the survival probability of autogenously transplanted canines over a period of 14 years.

Statistical analyses were performed with SAS (version 9.2) and S-plus (version 6.2). Significance was defined as $p<0.05$.

2. Results

The aim of the retrospective study was to determine the influence of different parameters (see: Material and methods, Table 1) on the long-term success rate of autogenously transplanted canines. From the patients who were willing to participate in this study, the different **preoperative** parameters were looked up in their files. Depending on the number of transplanted canines still present in the mouth, the **survival rate** was calculated. During a clinical and radiological examination of the transplanted teeth various **postoperative** parameters were evaluated in order to determine the long-term **success rate**. In addition, we searched for a statistic model containing all preoperative parameters which could be explanatory in determining the success rate of autotransplantation.

2.1 Selected patients and preoperative parameters

Out of 133 patients, who had undergone canine transplantation from 1995 until 2002, 60 patients (74 teeth) volunteered to participate in this study. Consequently, the patient response rate was 45.1%. Thirty-five patients (42 teeth) were males and 25 patients (32 teeth) were females (Table 3). The patients ranged in age from 11 to 46 years (mean 21 years, Table 3). An important characteristic of this study is its **long-term follow-up**. The follow-up period varied from 6 to 14 years with a mean follow-up time of 11 years (Figure 6).

Table 3: The number of patients (n), the number of transplanted teeth (N) and the mean age at time of transplantation in years with the standard deviation (age/SD) subdivided by gender.

	N	*N*	*Age/(SD)*
Male	35	42	21.2/(9.9)
Female	25	32	19.9/(9.2)
Total	60	74	20.6/(9.5)

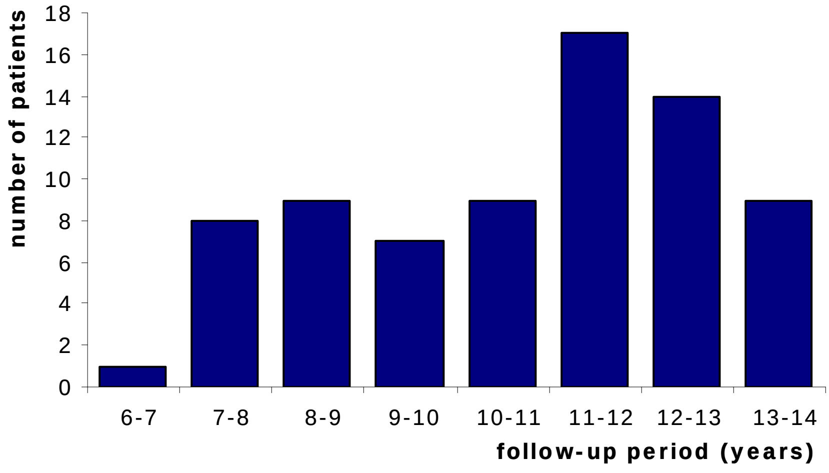

Figure 6: Distribution of the long-term follow-up period with a mean follow-up time of 11 years.

Out of the 74 transplanted teeth, 68 teeth (91.9%) were maxillary impacted canines while only three teeth (4%) were mandibular impacted canines. Two teeth (2.7%) were premolars from the mandibula transplanted to a canine position in the maxilla. One tooth (1.4%) was a premolar from the maxilla from one individual transplanted to a canine position in the maxilla from another individual (=allotransplantation). Furthermore, 66 of the 71 impacted canines (93%) were located palatal and the other five impacted canines (7%) were located labial. Fourteen impacted canines (19.7%) caused resorption of the root of an adjacent incisor. Seven canines (9.9%) were previously surgically exposed for orthodontic realignment, but this treatment plan failed.

At the time of transplantation four of the 74 transplanted teeth (5.4%) had a root which was 1/2-3/4 of the final root length, 16 teeth (21.6%) had a root >3/4 of the final root length and 36 teeth (48.7%) had a complete root. In 18 cases (24.3%) it was not possible to document the root length of the transplanted teeth. Eighteen transplanted teeth (24.3%) showed an open apex, 38 teeth (51.4%) showed a closed apex and from the remaining 18 teeth (24.3%) the status of the apex could not be evaluated. An apical anomaly could be detected in 20 of the 71 impacted canines (28.2%) meaning they had a curved apex. Out of 60 patients, who participated in this study, 14 patients (23.3%) suffered from bilateral displaced canines at time of transplantation.

In 69 cases (93.2%) there was sufficient space for transplantation. During transplantation, the PDL of only 12 teeth (16.2%) was damaged. However the condition of the PDL was not recorded in the operation files for 29 teeth (39.2%). All transplants, except one, were stabilized with either a trauma splint (23%) or an orthodontic wire (75.7%). In addition, more than half of the transplanted teeth (55.4%) were fixed in infra-occlusion. Four teeth (5.4%) were transplanted in an occlusive position. The occlusion state of the other transplanted teeth (39.2%) could not be traced in the patients' files.

Taken together, these data indicate that the preoperative parameters are not equally distributed and that for some teeth a number of parameters are missing.

2.2 Survival rate

Before examination already 18 transplanted teeth were lost due to extraction, these teeth were recorded as failures and taken into account to calculate the survival rate of the transplantation. The survival rate was defined as the percentage of transplanted teeth still present at the examination (56 teeth) relative to the total number of teeth that were transplanted (74 teeth), which resulted in 75.7%. Figure 7 represents the Kaplan-Meier risk curve for the overall survival rate over a period of 14 years. One transplant was extracted six months post-operatively because of a delayed endodontic treatment. The other transplanted teeth were extracted 4 (n=2), 5 (n=1), 6 (n=1), 7 (n=5), 8 (n= 3), 9 (n=1), 10 (n=1), 11 (n=1) and 12 years (n=2) after surgery. The most common reason for failure was progressive root resorption, which caused the loss of nine teeth. Figure 8 shows a clinical example of a transplanted canine which was lost due to progressive root resorption. In conclusion, the chance that a transplanted canine is still present in the mouth 14 years after transplantation is 75.7%.

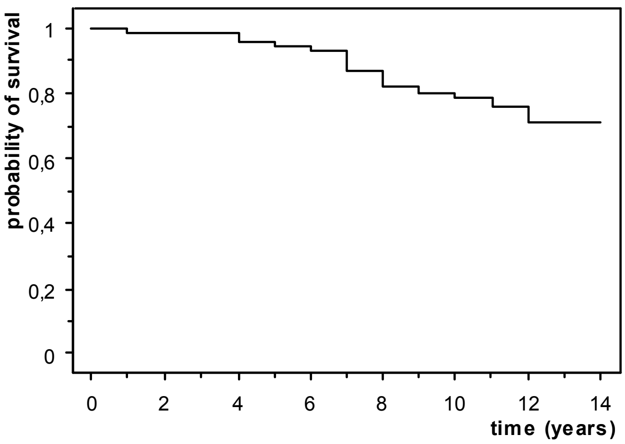

Figure 7: The Kaplan-Meier risk curve plots the probability of survival as a function of time (years).

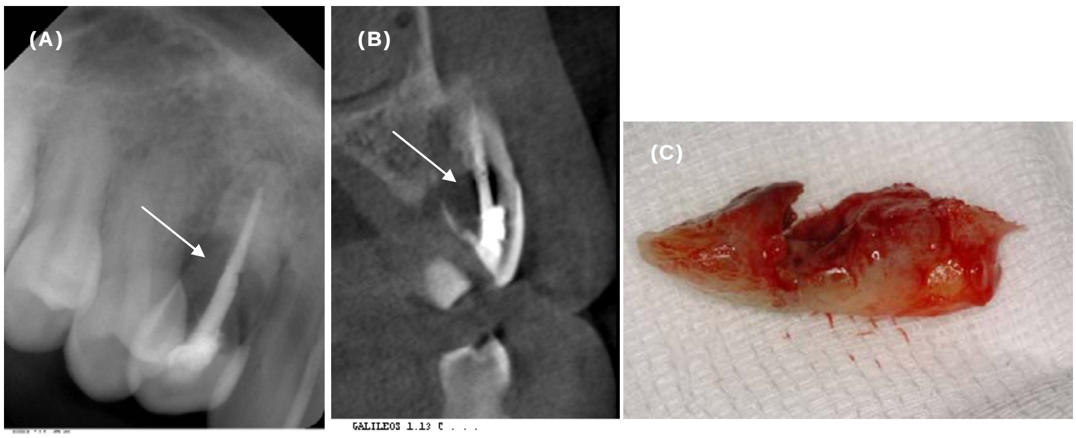

Figure 8: A periapical x-ray (A), a cone-beam volumetric cross section (B) and a clinical picture showing a tooth with progressive root resorption (white arrows).

2.3 Postoperative parameters

Because 18 teeth were already extracted before the examination, a total of 56 transplanted teeth were clinically and radiologically assessed during this study.

2.3.1 Tooth mobility

The periotest was used to assess the mobility of the transplanted tooth. Overall 36 transplanted teeth (64.3%) showed a periotest value which was lower than the normal values meaning the teeth lost mobility (=ankylosis). On the other hand, two teeth (3.6%) were clinical mobile and displayed a periotest value higher than the normal ranges. The remaining 18 teeth (32.1%) had a normal periotest value.

2.3.2 Tooth vitality

Endodontic treatment was needed in 22 of 56 examined teeth (39.3%) after transplantation. Tooth vitality was only tested in teeth which were not endodontic treated (34 teeth). Vitality of the transplanted tooth was examined by means of a cold-test, an electric pulp test and a laser Doppler flowmeter. Results are shown in Table 4. Out of 34 transplanted teeth only one (2.9%) showed a positive result for the cold-test and five transplanted teeth (14.7%) showed a positive result for the electric pulp test. The laser Doppler flow graphs illustrated a clear pulsatility for 26 of the 34 transplanted teeth (76.5%). An example of a laser Doppler flow graph is shown in Figure 9.

Table 4: The results for the different vitality tests are shown in this table.

Vitality	***positive***	***negative***	***total***
Cold test	1	33	34
electric test	5	29	34
LDF	26	8	34

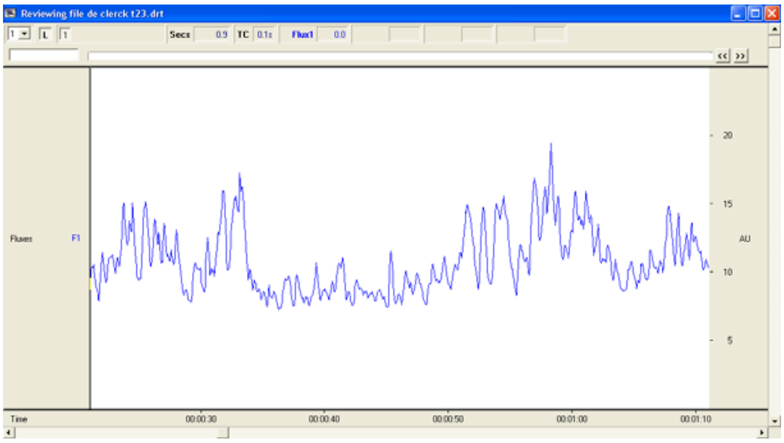

Figure 9: An example of a laser Doppler graph showing a good pulsatile blood flow that varies with time.

2.3.3 Pocket depth and inflammation

Good gingiva attachment occurred only with sufficient regeneration of the alveolar bone. In cases of insufficient bone regeneration, pathological pockets (>3mm) between the tooth and the surrounding gingiva were formed. Overall 11 transplanted teeth (19.6%) showed an unacceptable clinical pocket depth. Figure 10 illustrates a particular clinical case of massive recession of the gingiva due to periodontal breakdown of the vestibular bone. This is an example of a clinical failure but a positive survival because the tooth is still present in the mouth 12 years after transplantation.

Thirteen transplanted teeth (23.2%) showed moderate inflammation, meaning that the gingiva bled while probing the pocket depth.

Figure 10: Clinical picture of an upper canine in the left jaw showing massive recession of the gingiva due to periodontal breakdown of the vestibular bone.

2.3.4 Colour

Fifty-three transplanted canines (94.6%) showed a significant difference in colour compared with non-transplanted canines. In only 3 of 56 canines (5.4%) no discoloration was found after transplantation, the average deltaE*ab values were below 5. A high standard deviation was found between the five deltaE*ab values of each tooth.

2.3.5 Resorption

Twenty-two transplanted teeth (39.3%) showed one or other form of root resorption (see: Introduction, Figure 3) and 34 teeth (60.7%) showed no resorption at all. Twenty transplanted teeth (90.9%) showed external root resorption while only two teeth (9.1%) showed internal root resorption. One specific radiological case of a transplanted canine, which showed external root resorption, more specifically replacement resorption, is presented in Figure 11. Figure 11A illustrates replacement resorption of the transplanted canine in the right upper jaw on a periapical x-ray. Cross sections of a cone-beam volumetric scan through the same transplanted canine are shown in Figure 11B and C, where the entrance of the alveolar bone in the tooth becomes clear. In Figure 12 a particular case is shown in which the diagnosis based on the periapical radiograph (Figure 12A) differs from the diagnosis based on the cone-beam scan (Figure 12B). On the periapical radiograph the apical root contours of the transplanted canine were not clear suggesting there is some apical resorption. The cone-beam scan, however, showed that there was no root resorption and that the tooth is placed outside the alveolar socket.

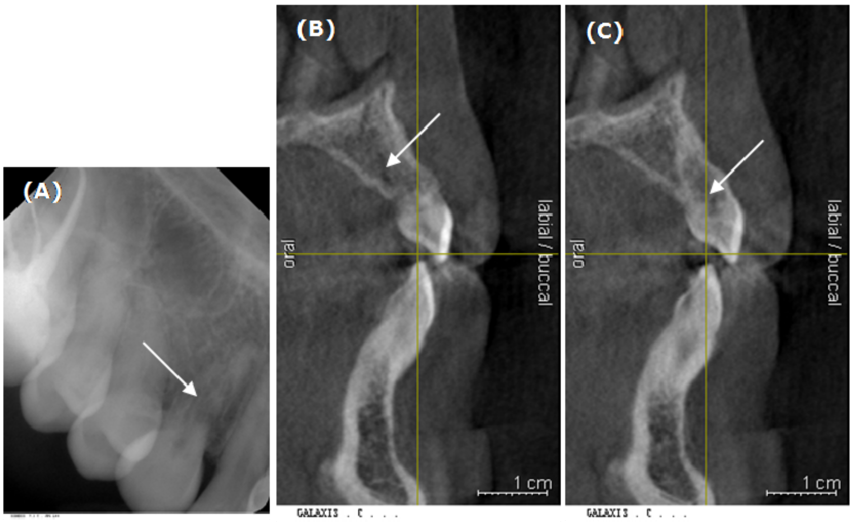

Figure 11: (A) Periapical x-ray of a transplanted canine in the right upper jaw showing replacement resorption (white arrow). (B, C) Cross sections of a cone-beam volumetric scan (Galileos, Sirona, Bensheim, Germany) through the same transplanted canine showing root resorption (white arrows). The first cross section (B) shows the entrance of the alveolar bone in the tooth.

Figure 12: (A) Periapical x-ray of a transplanted canine in the left upper jaw, the apical root contours of the tooth are not very clear (white arrow). (B) A cross section of a cone-beam volumetric scan through the same tooth, the white arrow indicates the alveolar socket.

2.3.6 Pulp obliteration and lamina dura formation

Pulp obliteration can only be established in teeth without an endodontic treatment (34 teeth). In 15 of the 34 teeth (44.1%) there were no important changes in the pulp chamber. Other teeth, excluding endodontic treated teeth, showed a reduction in size or a complete obliteration of the pulp chamber. Figure 13 illustrates a transplanted canine in the left upper jaw showing pulp obliteration. From the 56 transplanted teeth who survived until the follow-up, 29 transplanted teeth (51.8%) showed an intact lamina dura. In 16 teeth (28.6%) no lamina dura could be detected on the radiographs. The remaining 11 teeth (19.6%) only missed a part of the lamina dura.

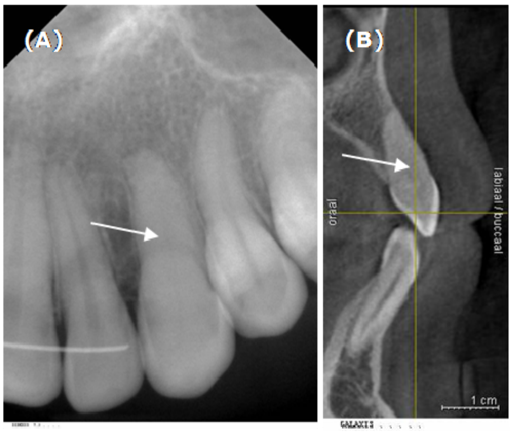

Figure 13: Transplanted canine in the left upper jaw showing pulp obliteration on (A, white arrow) a periapical x-ray and on (B, white arrow) a cross section of the cone-beam volumetric scan (Galileos, Sirona, Bensheim, Germany).

2.3.7 Alveolar bone loss and apical inflammation

Out of the 56 transplanted teeth which were examined, six teeth (10.7%) showed extensive alveolar bone loss after transplantation. A clinical example is shown in Figure 14A. Figure 14B illustrates a clinical case of apical inflammation. Of all transplanted teeth, only three (5.4%) exhibited an apical inflammation.

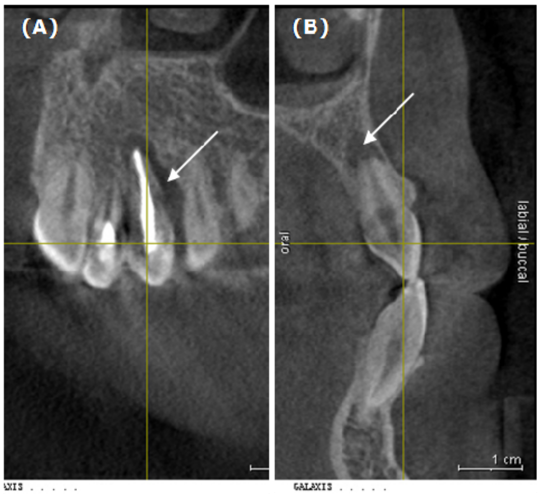

Figure 14: Cross sections of a cone-beam volumetric scan (Galileos, Sirona, Bensheim, Germany) showing a clinical case of a transplanted canine with alveolar bone loss (A, white arrow) and one with apical inflammation (B, white arrow).

In general, cone-beam volumetric images gave a better insight in the examined radiological parameters compared to conventional two dimensional radiographic images.

2.4 Success rate

Overall 43 transplanted canines were evaluated as clinically successful, meaning they exhibited no progressive root resorption, no pathological pockets, no extensive loss of alveolar bone nor an apical inflammation. The success rate was defined as the percentage of clinically successful transplanted teeth (43 teeth) relative to the total number of transplantations in the sample (74 teeth), which resulted in 58.1%.

2.4.1 Univariate analysis

The results of the univariate logistic regression analysis are shown in Table 5. Besides age at transplantation, which was a continuous covariate, all other pre- and peroperative parameters were categorical covariates. The only parameters found to be statistic significant with respect to success rates in our study was age at transplantation. The success rate significantly decreased when the age at transplantation increased (OR, 0.95; CI, 0.90-0.99; $p=0.0312$).
The influence of other pre- and peroperative parameters on the success rate was not found to be statistically significant (Table 5). The success rate of canine autotransplantations for male patients was 47.6%, whereas it was 65.6% for female patients ($p=0.2508$). While the success rate of autotransplantations performed on palatal impacted canines was 57.6%, it was 40% for autotransplantation performed on labial impacted canines ($p=0.4469$). Important to consider, only five impacted canines were located labial. The success rate of impacted canines which were previously surgically exposed was 42.9% compared to 59.7% for not surgically exposed canines ($p=0.3938$). Again, it has to be considered that only seven of the 74 transplanted teeth were previously surgically exposed. The success rate was lower for teeth transplanted in a too small diastema (40%) than for transplanted teeth which had enough space (59.4%). However, this difference was not statistically significant ($p=0.3992$). No significant difference in success rate was

found between teeth transplanted at a different root development stage (p=0.4490). For teeth transplanted with an open apex, the success rate was 72.2% which was not significantly higher than the success rate (60.5%) for teeth transplanted with a closed apex (p=0.3883). In addition, the success rates for impacted canines with a curved apex and impacted canines without a curved apex were not significantly different (p=0.4618). Uni- and bilateral transplantations showed almost similar success rates, respectively 58.7% and 57.1% (p=0.8956). The success rate of transplantation of teeth who caused root resorption to adjacent teeth was 71.4%, whereas it was 55% for teeth who caused no root resorption (p=0.2537). When the PDL was not damaged during surgery 62.7% of the transplantations were successful compared to a success rate of 50% when the PDL was damaged (p=0.4643). For patients who had orthodontics 62.5% of the transplants were successful, while the success rate for transplanted teeth fixed with a splint was 41.1% (p=0.1209). The success rate for teeth placed in infra-occlusion after transplantation was 58.5%, while it was 25% for teeth placed in occlusion. This difference was not statistically significant (p=0.1935).

Table 5: Variables which could have an influence on the overall success rate of transplantation derived from the univariate analysis. OR: odds ratio. CI: confidence interval. Ref: reference.

Covariate (parameter)	**OR (95% CI)**	**Univariate p-value**
Patient:		
- Age at transplantation	0.95 (0.90-0.99)	**0.0312**
- Gender *female*	1.00 (Ref.)	0.2508
Male	0.58 (0.22-1.49)	
Transplantation area:		
- Position *palatal*	1.00 (Ref.)	0.4469
labial	0.49 (0.08-3.14)	
- Surgical exposure *no*	1.00 (Ref.)	0.3938
yes	0.51 (0.10-2.44)	
- Space *no*	1.00 (Ref.)	0.3992
yes	2.20 (0.34-14.00)	
Transplanted canine:		
- Root development *complete*	1.00 (Ref.)	0.4490
>3/4	2.14 (0.58-7.95)	
1/2-3/4	2.14 (0.20-22.65)	
- Apex *open*	1.00 (Ref.)	0.3883
closed	0.59 (0.17-2.00)	
- Curved apex *no*	1.00 (Ref.)	0.4618
yes	1.49 (0.51-4.31)	
- *Uni*lateral	1.00 (Ref.)	0.8956
*Bi*lateral	0.94 (0.36-2.43)	
Adjacent teeth:		
- Root resorption *no*	1.00 (Ref.)	0.2537
yes	2.05 (0.58-7.26)	
Pre-/perioperative:		
- Damage PDL *no*	1.00 (Ref.)	0.4643
yes	0.62 (0.17-2.22)	
- Fixation *splint*	1.00 (Ref.)	0.1209
orthodontics	2.38 (0.79-7.20)	
- Occlusion	1.00 (Ref.)	0.1935
Infra-occlusion	4.24 (0.41-44.27)	

2.4.2 Multivariate analysis

After the prognostic significance of each parameter was determined by univariate analysis, assessment of the relative importance of the parameters in explaining the success rate of canine autotransplantation was established by multivariate logistic regression analysis. The multiple regression models were fitted using all parameters with $p<0.25$ on the univariate analysis and those thought to be important on logical and medical grounds. The model which fitted the best to the data contained three preoperative parameters as covariates (Table 6). The significant parameter in determining the success rate of autotransplantation was age at transplantation ($p=0.0458$). More specifically, the age at transplantation had a negative correlation with the success rate (OR, 0.98; CI: 0.96-0.99). The higher the age at transplantation, the lower the success rate (Figure 15). Other parameters were not significant, including root development ($p=0.7730$) and condition of the apex ($p=0.5456$).

Table 6: The final model, derived from the multivariate analysis, contains three parameters. The parameter age at transplantation is explanatory for determining the success rate after autotransplantation of canines. OR: odds ratio. CI: confidence interval.

Covariate (parameter)	OR	CI (95%)	p-value
Age at transplantation	0.98	0.96-0.99	**0.0458**
Root development *>3/4* *1/2-3/4*	1.18 1.20	0.74-1.89 0.60-2.37	0.7730
Closed apex	1.16	0.72-1.88	0.5456

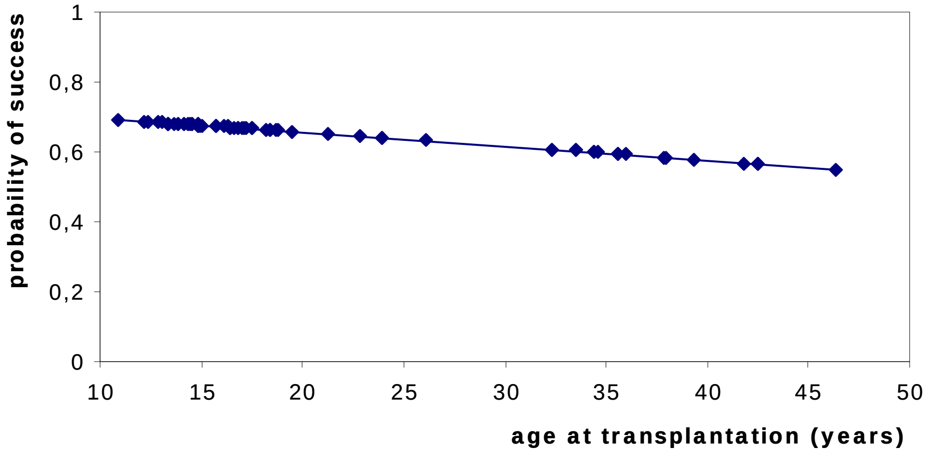

Figure 15: Probability of success in function of age at transplantation (years). The older the patient at the time of transplantation, the lower the probability of success.

2.4.3 Inter-examiner agreement

According to the simple κ statistics computed to determine the inter-examiner agreement on radiological parameters, we can conclude that the inter-examiner agreement for resorption is high and statistically significant (Table 7). There was no discordance between examiners in the evaluation of lamina dura formation and pulp obliteration.

Table 7: Simple κ statistics for inter-examiner agreement. /: no discordant pairs. CI: confidence interval.

Radiological parameter	**Simple κ**	**CI (95%)**	**p-value**
Resorption	0.6866	0.5084-0.8647	**<0.0001**
Lamina dura formation	/		
Pulp obliteration	/		

3. Discussion

Impacted canines cause great problems in treatment planning and are a specific reason for referral to both the orthodontist and the oral surgeon. The traditional treatment for ectopically positioned canines is surgical exposure and orthodontic realignment. When the position of the canine is not such that orthodontic treatment would be feasible, autotransplantation of impacted canines to their normal position in the dental arch is considered as an alternative. Because impacted canines are a particular challenge in children and adolescents, the transplanted tooth should preferably adapt to growth and developmental changes in the oral region. In addition, the tooth should have the potential for long-term, even lifelong survival. Many studies have already investigated the survival and success rate of autogenously transplanted premolars and molars. However, literature contains only a few long-term follow-up studies about transplanted canines. The present study focused specifically on the long-term survival and success rates of transplantation of impacted canines.

The overall response rate (45.1%) was satisfactory with a mean follow-up period of 11 years in mind. The transplanted teeth that were examined are considered to be representative of the long-term outcome of tooth transplantation in the total group of patients. The reasons for not taking part in this study appeared not to be related to any negative appreciation of the patient for the past transplantation. In contrary, the reasons were more of a secondary kind such as patients who moved to another country or patients who were occupied at the time of recall.
From the patients who were willing to participate, different pre- and peroperative parameters (see: Material and methods, Table 1) were looked up. Previous radiographs were used to determine the root length and the condition of the apex of each transplanted tooth. Eleven years ago only conventional radiographic methods such as periapical and panoramic radiographs were available. Due to superimposition on these 2D films, it was not always possible to document the developmental stage of the impacted canine. In recent years, 3D CBCT has been developed. These devices use cone-shaped radiation to gather information in the maxillofacial region, with high spatial resolution and significantly decreased radiation doses [30]. To date, the radiological preoperative parameters can be better assessed with accurate 3D imaging resulting in less missing data for future studies.

In the present study, a distinction was made between the survival rate and the success rate. In general, the survival rate is calculated as the percentage of transplanted teeth still present at the examination relative to the total number of teeth that were transplanted. The survival rate of transplanted canines in this study was 75.7%. It appears from other studies that tooth survival after autotransplantation of canines ranges from 86% to 100% [31-36]. The dissimilarity can be explained by the difference in follow-up period. An important factor in tooth survival assessment is the length of the observation period [6]. The follow-up period in our study ranged from 6 to 14 years, with a mean of 11 years. In contrast, the mean observation period in the other studies was only 5 years or even less. It has been proven that with increasing time after transplantation, significantly more root resorption is found [6]. Because progressive root resorption was the main reason of tooth loss in this study, this could be the explanation why less transplanted teeth survive for 11 years than for 5 years. Taken together, the present retrospective study showed a high survival rate of 74 transplanted canines observed with a mean follow-up period of 11 years.

To determine the success rate of the transplanted teeth, various postoperative parameters were evaluated during a clinical and radiological examination.

The diagnosis of pulp vitality depends both on the clinical results of sensibility tests (cold and electric pulp tests) and on the radiographic evidence of pulp obliteration. In addition, a distinction can be made between the reinnervation and the revascularisation of the teeth. Thermal and electrical pulp tests were applied to determine the innervation of the teeth, while LDF was used to assess the vascularisation of teeth. The results of tooth vitality indicated that 17.6% of the transplanted canines responded positive to the sensibility tests. This corresponds with other studies which show that between 11% and 43% of transplanted canines regain vitality after transplantation [33-36]. When using sensibility testing as an indicator for pulp vitality in a long-term follow-up study, it is important to consider that some teeth lose their sensibility response over time [6].

Obliteration of the pulp is related to ingrowth of connective tissue of the PDL into the pulp chamber [37]. Therefore, pulp obliteration is an important criterion for determining the vitality of tissues in the pulp chamber. In the present study, partial or complete pulp obliteration was radiographic detected in 55.9% of the transplanted canines without endodontic treatment. In harmony with our study, Andreasen et al. (1990) stated that the number of vital pulps is larger if pulp obliteration instead of sensibility tests is considered to be the decisive factor for evaluating pulp vitality [6].

LDF showed a pulpal blood flow in 76.5% of the transplanted teeth without endodontic treatment. This is rather a surprising result because positive LDF results are rare when pulp obliteration can be radiologically visualized. Obliteration caused by trauma decreases the pulp volume and thickens the hard tissue (bone and cementum), making the detection of pulpal circulation very difficult [38]. Our results may be explained by the fact that LDF signals obtained from the transplanted teeth may not only indicate pulpal blood flow. The contribution of tissues other than pulp such as lips, tongue, PDL or gingiva should be considered [39]. Further research is necessary about the use of LDF meters in assessing the vitality of teeth.

In the present study, colorimetry was used as a new technique to assess possible colour difference between a transplanted canine and a non-transplanted canine. However, this technique has still some limitations. The colour difference was calculated from five different locations on the transplanted tooth. The deviation between the resulting 5 deltaE*ab values was high, possibly because the tooth itself already possesses many colour differences. In the future, this technique needs to be further optimized in order to define uniform locations on each tooth in each patient.

The irreversible process, in which the root of the transplanted tooth is resorbed and replaced by bone, is called ankylosis. Ankylosis can be diagnosed both clinically by little to no mobility of the tooth and radiographically by the lack of lamina dura. The mobility of the transplanted tooth was tested by means of the periotest. Our results showed that 64.3% of the transplanted teeth had a periotest value lower than normal, indicating ankylosis. Radiographically, however, the lamina dura was partly or completely absent in 48.2% of the transplanted teeth. This difference can be explained by the fact that radiographic visualisation of ankylosis requires involvement of at least 40% of the root surface [6].

In general, cone-beam volumetric images gave a better insight in the examined radiological parameters compared to conventional 2D radiographic images. Our results showed two clinical cases in the diagnosis of resorption. In a first case, the diagnosis based on a periapical radiograph and on cross-sections of a cone-beam volumetric scan was the same. Both showed that the root of the transplanted tooth was replaced by bone (=replacement resorption). However, on the 3D images of the cone-beam scan the entrance of the alveolar bone in the tooth could be clearly seen. In a second case, the diagnosis differed between the 2D and 3D radiographic images. The conventional periapical radiograph showed some apical root resorption while the cone-beam CT showed no root resorption. However, based on the 3D cone-beam images it seemed that the tooth is slightly outside its alveolar socket. The reason for the discrepancy remains unclear. A possible explanation may be the position of the tooth, which could lead to superimposition on the periapical radiograph. Taken together, CBCT provides an added value in the follow-up of tooth transplantation.

During the clinical and radiological examination, 43 transplanted canines were evaluated as clinically successful resulting in a success rate of 58.1%. A clinically successful transplantation was achieved when a tooth had no progressive root resorption, no pathological pockets, no alveolar bone loss nor apical inflammation. When comparing the success rate of this study with other success rates in literature, it is important to consider the difference in criteria for success because there are no common success criteria. A remarkable difference with other studies is that in the present study transient root resorption was not considered as a criterion for unsuccessful transplantation because even in cases of eventual failure, autotransplanted teeth may be retained for considerable lengths of time providing the patient with an aesthetic and functional solution [7].
The success rate of transplanted teeth in this study, 58.1%, is lower than the success rate of transplantation reported in other studies (~90%) [37, 40-42]. This can be explained by the fact that most studies included all tooth types in their calculation of the success rate of the transplantation procedure. However, some studies also mention the specific success rate for canine transplants. Pogrel et al. (1987) found a success rate of 62% for the transplantation of maxillary canines after a follow-up of at least 2 years [43]. In the study of Kallu et al. (2005) the success rate for canines was 51% with a mean follow-up time of 3.8 years [8]. These rates are comparable with the success rate which is found in the present study, but our results indicate the success rate 11 years after surgery.
There are multiple reasons why the success rate of canines is lower than the success rate of other tooth types. Impaction of the canines is an important factor that influences the outcome of transplantation [7]. The accessibility of the tooth during surgery is less so that the chance of damaging the root surface while extracting is higher. In addition, the PDL in impacted teeth is thinner than in teeth that are functioning normally [9]. Furthermore, when transplanting impacted canines to their normal position there is not always a preformed alveolus for positioning the donor tooth. It is already proven that autotransplantation is more successful if the donor tooth is transplanted to an extraction site with PDL attachment still present in the recipient socket [7, 16]. Therefore, success rates of teeth transplanted into artificially formed sockets are lower than of teeth transplanted into extracted sockets [16]. A reason for this may be the extended extra-oral time of the donor tooth when forming a recipient socket and the increased PDL damage due to

socket adjustment. A group of researchers already suggested the use of a computer to produce a duplicate form of the donor tooth before extraction [13, 23]. Consequently, it would be possible to prepare the recipient socket with the template instead of the donor tooth thereby reducing damage to the donor tooth. In this way, using a tooth transplantation template might have an influence on the success rate of autotransplantation. The prospective study of this report focuses on the construction of a tooth transplantation template.

To determine the influence of various pre- and peroperative parameters on the success rate of autotransplantation of canines, logistic regression analyses were performed. To our knowledge, no such study has been previously carried out.
The significant parameter in determining the success rate of autotransplantation was the age at transplantation (see: Results, Table 5). More specifically, the age at time of transplantation had a negative correlation with the success rate, meaning the younger the patient the higher the success rate. This result may be explained by the better healing capacity in younger patients. Corresponding with our results, Hovigna (1969) indicated age as an important factor in determining the prognosis of a canine transplant. It appeared that transplantation before the age of 20 was more successful than at an older age [44]. However, based on our study no particular age or age group could be selected in which canine transplantation showed the best results. It is worth to consider this topic in future studies.
All other pre- and peroperative parameters had no statistically significant influence on the long-term success rate of canine transplantation. However, we expected an influence on the success rate for some of these parameters. Based on literature, the maintenance of the healthy PDL cells is an important consideration for a successful tooth transplant [14, 16]. The correlation between damage to the PDL and the success rate of transplantation could not be verified in the present study. A possible explanation could be that during extraction there is always some degree of damage to the PDL and it is difficult to determine the exact degree of PDL damage. It is not possible to be sure that the entire PDL is vital or undamaged after extraction of the donor tooth.
From the literature it is known that root shape and maturity of the root structure of the donor tooth are influencing factors for the prognosis of autotransplantation [6, 7, 45]. However, a lower success rate due to a curved apex could not be established in this study. In addition, no significant difference in success rate was found between the various root developmental stages. The main reason why these and possibly other preoperative parameters were not found to have a significant influence on the success rate of canine transplantation is because of missing data and unbalanced data.

When an impacted canine is positioned such that orthodontic repositioning is not possible there are only two treatment options. The impacted canines can be transplanted to their normal position in the dental arch or they can be extracted and implant techniques may be used to restore the edentulous area. In clinical practice, it makes sense to maintain the use of natural teeth for as long as possible. Moreover, tooth transplantation offers several benefits compared with dental implants in terms of time, function, aesthetics and cost. A tooth transplantation procedure can be performed in a single surgery. After transplantation, the transplanted tooth restores its function and normal periodontal healing. Prosthetic treatment is not required in most of the transplanted teeth, and

natural aesthetics can be maintained. Furthermore, implant treatment is about ten times more expensive than transplantation [7, 16, 46]. Implants are not indicated in patient before bone growth has been completed. In contrast, tooth transplantation in growing patients can offer the benefit of continued alveolar bone induction [14].
Taken together, even if the transplantation turns out to be unsuccessful, autotransplantation was still the best treatment option and dental implants could be at least delayed.

Due to the retrospective aspect of this study, the main limitation was the availability of information in the medical files when so many parameters are taken into consideration. Despite this limitation, the present study has demonstrated that transplantation of impacted canines may have a successful outcome 11 years after transplantation. Moreover, the age at transplantation was found to be an important prognostic factor. In the future, these results could be improved by adding more patients and collect pre- and peroperative parameters as complete as possible. Furthermore, it is recommended to have a group of patients with an approximately equal distribution for each parameter.

Prospective study

1. Material and methods

Computer-aided rapid prototyping can be used to construct a tooth transplantation template and consists of three major steps (see: Introduction, Figure 4). The present study focused on image segmentation. Three different segmentation methods, which are available at the department OMF of the St. John's Hospital Genk, were compared to determine which segmentation method is the most suited for tooth segmentation. The most appropriate segmentation method was used for implementation in a case study. A detailed description of the selected segmentation method is included in Supplemental Section 3.

1.1 Data collection through cone-beam computed tomography

The material of this study consisted of six dry human skulls, which were obtained with approval from the Institute of Biomedical Research (BIOMED, University Hasselt, Belgium). Three-dimensional data (DICOM format, Digital Imaging and Communication in Medicine) of the dry human skulls were obtained using the cone-beam scanner Galileos (Sirona, Bensheim, Germany). All scans were performed at 85kV and 7mA according to the routine clinical procedure applied at the department OMF of the St. John's Hospital Genk. The Galileos device features a fixed field of view of 15cm resulting in a scan volume of 15cm x 15cm x 15cm. To better simulate soft-tissue, the dry skulls were wetted by placing them in a plastic box filled with water (Figure 16 *left*). Out of these six dry human skulls, ten molar teeth were randomly chosen to segment. We preferred to segment molars because they are the most commonly transplanted teeth, especially third molars [6]. After scanning the skulls, the chosen molars were extracted and scanned again with the same scanning parameters (Figure 16 *right*). The segmentation result of the extracted teeth was used as a standard reference. To determine which segmentation method was the most suitable, the segmentation result of the tooth in the maxilla was compared with the segmentation result of the single tooth.

Figure 16: A dry human skull (*left*) and a single tooth (*right*) placed in a box filled with water to better simulate soft tissue.

1.2 Data processing via software programs

The 3D data obtained from the Galileos were processed by means of image segmentation. Segmentation of a 3D image means that a label is assigned to each voxel in the image such that voxels with the same label share a certain property (e.g. intensity). For this purpose, segmentation algorithms integrated in software programs are used.

In this study thresholding, edge-based and region-based algorithms were examined. These algorithms are included in the software programs Amira 4.1 (Visage Imaging) and ITK-SnAP 1.6. Amira was applied to segment the tooth with thresholding, while ITK-SnAP was used to segment the tooth with region-based or edge-based algorithms. In this study, the semi-automatic mode of the ITK-SnAP software was applied, which means that automatic segmentation was followed by manual checking and editing. DICOM data were exported from the Galileos software to the Amira or ITK-SnAP software. After segmentation the results were saved as STL files. The STL format is the standard input format for the rapid prototyping machine.

Segmentation was performed on the 3D image of the tooth in the maxilla and on the 3D image of the extracted tooth. In order to compare the two resulting images, the same segmentation algorithm was used. Comparison between both images was made with the software program Amira. Therefore, the surfaces of the two images were aligned and the surface distance between them was calculated. By doing this for every segmentation algorithm separately (Figure 17), the segmentation algorithm which led to the smallest surface distance between the tooth in the maxilla and the extracted tooth was determined. In addition, the time needed to segment the tooth from the maxilla was recorded for each segmentation method. Based on the surface distance and the segmentation time, the most appropriate method for tooth segmentation was chosen.

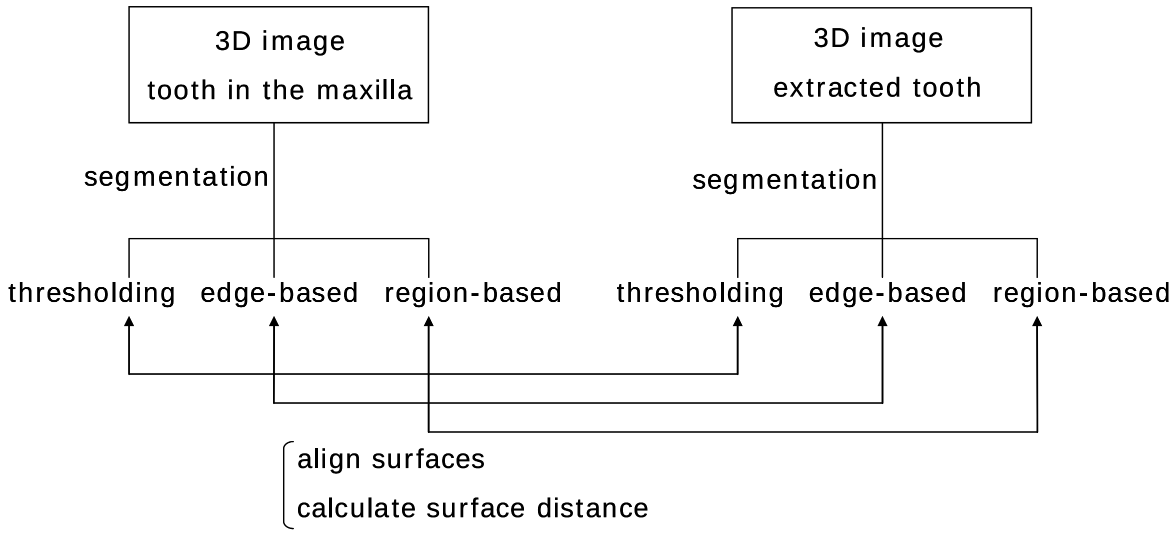

Figure 17: Study design. 3D images of the tooth in the maxilla and the extracted tooth were both segmented with the same method and then the surfaces were aligned and the surface distance was calculated.

1.3 Fabrication of the tooth transplantation template

For fabrication of the template in the case study, the STL files were outsourced to SKM Rapid Modelling B.V. (Helmond, Netherlands). The tooth model was printed out by the STL technique. STL is a rapid prototyping technology based on a bath of photosensitive liquid (see: Supplemental section 4) which becomes cured when exposed to a laser beam [21]. There is a platform in the liquid which descends by a single slice thickness and so the model is built layer by layer (Figure

18). After printing, the template was submitted to a quality control and further modified by a technician in the digital dental laboratory of Aporis nv (Diepenbeek, Belgium).

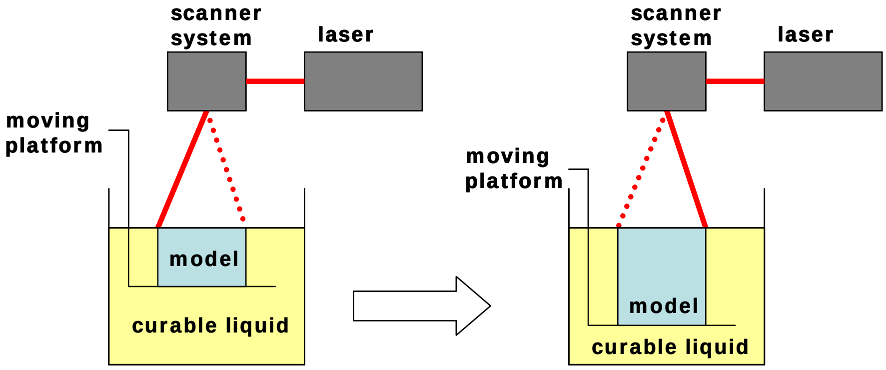

Figure 18: Schematic figure showing the principle of operation of a STL system.

1.4 Statistic analysis

A paired t-test was performed to determine if there was a significant difference in surface distance and segmentation time between the different segmentation methods. Values were reported as the mean ± SD. Differences in mean values were considered significant if p-value < 0.05. Analyses were performed with StatView, version 5.0.

2. Results and discussion

2.1 Image segmentation

In order to optimize the protocol for the production of a tooth transplantation template, a comparison was made between three different segmentation methods. The first segmentation method was thresholding. **Thresholding** is the simplest method of image segmentation, it creates binary images from grey-level ones by turning all voxels below some threshold to zero and all voxels above that threshold to one [47]. Thresholding was performed on the 3D images of the teeth in the maxilla, but after trying on several teeth it became clear that the threshold-based method was not able to discriminate teeth from the alveolar bone. No threshold value could be found that resulted in an image of only the teeth without any alveolar bone. This can be explained by the fact that the bone regions have an intensity which is close to the intensity of teeth. In addition, the image intensity of a tooth is not homogeneously with enamel having the highest intensity and root having the lowest intensity. Figure 19 shows the result of using thresholding on randomly chosen teeth in the software program Amira. The study design (see: Figure 17) was to compare 3D images of teeth in the maxilla with 3D images of extracted teeth both segmented with the same method. Because it was not possible to segment the teeth from the maxilla using thresholding, it was of no meaning to perform thresholding on the 3D image of the extracted tooth. Therefore, the segmentation method thresholding was excluded from the study.

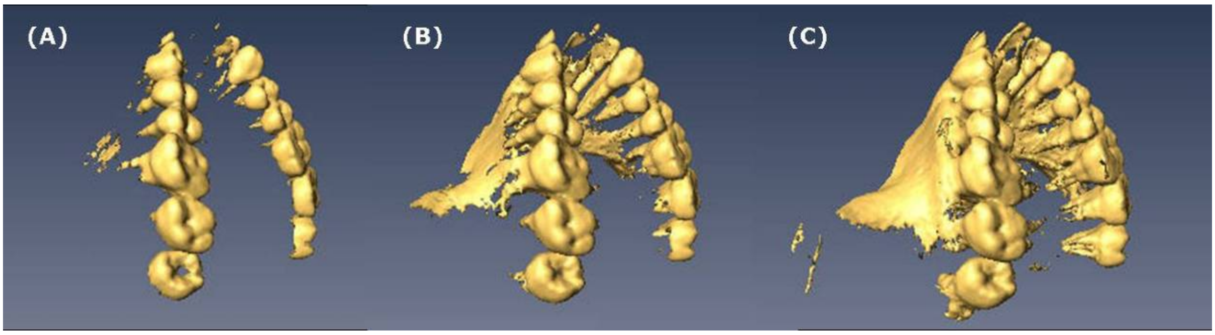

Figure 19: Tooth segmentation based on thresholding in the software program Amira. Threshold values used for the three images are 800 (A), 400 (B), 100 (C). No threshold value could be found to discriminate between the teeth and the alveolar bone.

The other two segmentation methods were both included in the software program ITK-SnAP**.** The **region-based** segmentation method tries to find coherent regions with similar pixel intensity [47]. The **edge-based** segmentation method, on the other hand, attempts to identify boundaries according to pixel differences [47]. The results of region- and edge-based segmentation on the same tooth are shown in Figure 20.

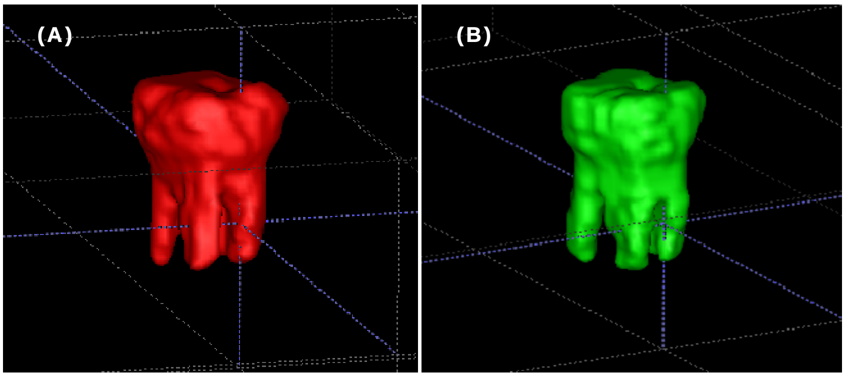

Figure 20: Segmentation based on intensity regions (A) and on image edges (B) of the same tooth in ITK-Snap.

Following, the Amira software was used for overlap analysis between the tooth segmented from the maxilla and the same tooth segmented after extraction. Overlap analysis calculates the surface distance between the two 3D images. In this way, it would be possible to determine which segmentation method results in a tooth transplantation template which resemble the real tooth most closely. Figure 21 shows how two 3D tooth images are placed on each other in order to calculate the surface distance.

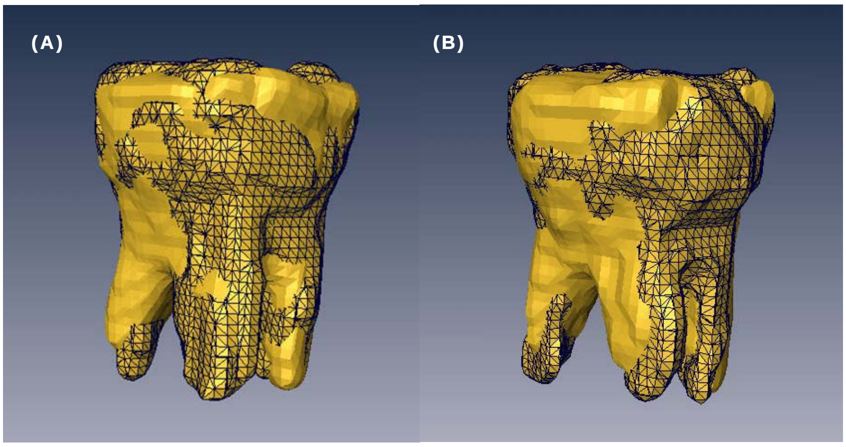

Figure 21: Overlap images of the extracted tooth (shaded draw style) and the tooth segmented from the maxilla (outlined draw style) for region-based (A) and edge-based (B) segmentation methods in the software program Amira.

After comparing the results of ten different molars, no significant difference in surface distance (p-value=0.63) was found between region- and edge-based segmentation (Table 8 and Supplemental section 5). The mean surface distance between teeth segmented from the maxilla and the segmentation results of the corresponding extracted teeth was 0,26mm (SD 0,05mm) for the region-based segmentation method and 0,25mm (SD 0,06mm) for the edge-based segmentation method (Figure 22). However, there was a significant difference (p<0.0001) in the time needed to complete the segmentation procedure (Table 8 and Supplemental section 5). The mean segmentation time for the region-based method was about 15 minutes (SD 7min), while an average time of 26 minutes (SD 8min) was required to finish edge-based segmentation (Figure 23). The standard deviations of seven and eight minutes can be explained by the fact that the segmentation time is dependent of the development stage of the tooth. Segmentation of teeth with fully developed roots lasted longer than the segmentation of teeth with undeveloped roots. Based on the difference in segmentation time, we can conclude that the most appropriate method for tooth segmentation is the region-based technique.

Table 8: Mean values ± SD of the surface distance (mm) and segmentation time (minutes) for the region-based and edge-based method. P-values are also shown.

	Region-based	*Edge-based*	***p-value***
Surface distance	0.26 ±0.05	0.25 ± 0.06	0.63
Segmentation time	15 ± 7	26 ± 8	**<0.0001**

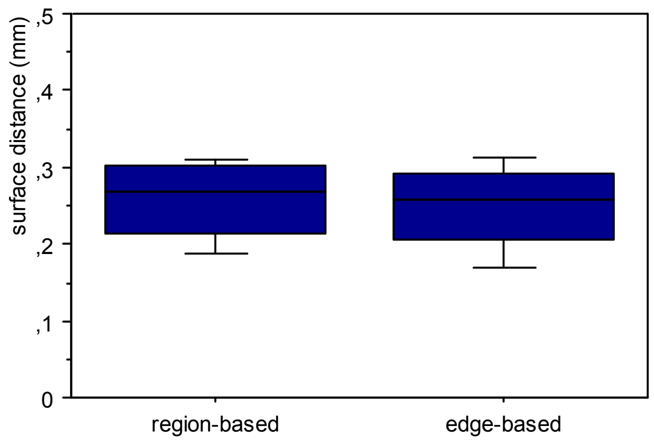

Figure 22: Surface distance between teeth segmented from the maxilla and the segmentation results of the corresponding extracted teeth both for the region-based and edge-based method presented in box plots.

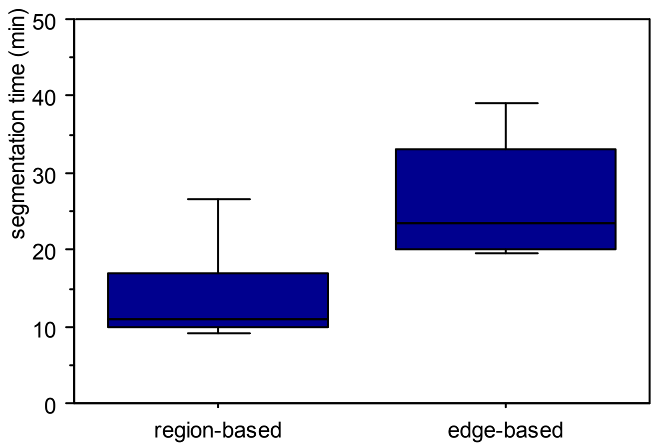

Figure 23: Box plots representing the total segmentation time for the region-based and the edge-based segmentation.

In this study, the semi-automatic mode from the ITK-SnAP software was used. This means that automatic segmentation based on either the region-based or edge-based algorithm is followed by manual checking and editing of the tooth boundaries [48]. The difference in time between the region-based and edge-based segmentation was directly proportional with the difference in manual editing between the two methods. The strategy of the edge-based segmentation method is to find pixels with a large intensity difference (='edges'). Because object boundaries are characterized by large intensity changes, segmentation based on edges requires the presence of a sharp boundary between the region of interest and the surrounding tissues. However, in 3D images no sharp edges are present between the teeth and the background. Hence, the edge-based technique was not able to accurately extract the tooth contour without manual editing. In contrast, region-based segmentation tries to find pixels with the same intensity but allowing some internal variation. Region-based segmentation makes use from an algorithm which takes into account the intensity of the whole tooth and not just the edges. Therefore, region-based automatic segmentation already led to a very smooth and accurate tooth contour. As a result, more manual editing was needed after edge-based segmentation of the images than after region-based segmentation.

The purpose of this study was to compare three existing segmentation methods in order to find the most suitable method for tooth segmentation. In other studies the same conclusions as in this study have been drawn. **First**, the present study illustrated that segmentation based on thresholding was not appropriate for tooth segmentation. Corresponding with this result, Akhoondali et al. (2009) showed that the global threshold method, which is commonly used in 3D reconstruction systems, cannot be applied for tooth segmentation [49]. In addition, Said et al. (2001) noticed that threshold-based methods fail to discriminate teeth from bone [50]. **Secondly**, in the present study more time was needed for edge-based tooth segmentation than for region-based tooth segmentation due to the fact that extraction of the tooth contours with the edge-based method was not as accurate as with the region-based method. Similarly, Shah et al. (2006) demonstrated that the tooth contour extracted based on edges did not fit tightly around the actual boundary of the teeth [51]. On the other hand, the contours that were extracted using a technique that depends on the intensity of the whole tooth region and not just the edges fitted the boundaries rather tightly [51].

In this study, CBCT was used to acquire 3D images of the teeth. However, in some other studies they used conventional CT to obtain images from the dental arch [13, 14, 23]. Liang et al. (2009) performed a study to compare the 3D model accuracy between five CBCT scanners (including Galileos) and one CT system. The results showed that CBCT has somewhat lower segmentation accuracy than CT but without any clinical significance [52]. Considering this in addition with the lower radiation, shorter scanning time and the comparable image quality [53] of CBCT in relation to CT, CBCT can be preferred in acquiring 3D data for the construction of a tooth transplantation template.

The accurate segmentation of teeth from 3D images is an important step in computer-aided rapid prototyping. Because teeth exist in different shapes and the arrangement of teeth varies between individuals, tooth segmentation is difficult. By determining which segmentation method was the most appropriate for tooth segmentation, the application of computer-aided rapid prototyping in constructing a tooth transplantation template was optimized. Making use of a tooth transplantation template might have an influence on the **success rate** of autotransplantation. Normally during tooth transplantation surgery the extracted donor tooth itself is used to contour the recipient alveolar bone in order to fit the donor tooth. Consequently, the PDL of the donor tooth might be injured unnecessary during a number of trials for fitting into the recipient socket. Computer-aided rapid prototyping makes it possible to construct a duplicate model of the donor tooth, which can be used for recipient bone contouring. Subsequently, the injury of the donor tooth during surgery will be minimized. Because the vitality of the PDL attached to the donor tooth is an important factor in influencing the success rate of autotransplanted teeth [16], using a template might increase the success rate of tooth transplantation. In this report, we present a case study in which a template was used during transplantation. But further research is necessary to determine the exact influence of using a template on the success rate of autotransplantation.

2.2 Case study

Missing anterior teeth in children are a particular challenge. Implants are not indicated because the replacement should preferably adapt to growth and developmental changes in the oral region. Autotransplantation of premolars, on the other hand, is a treatment plan that has received increasing attention in recent years.

Here, a case report of a 15-year old girl is presented on autotransplantation of a lower premolar to replace a maxillary incisor. The girl had a dental bridge at the position of the right upper central incisor. Because of a crowded mandibular arch, the left first premolar and the milk tooth on top of it need to be extracted. Therefore, it became possible to replace the dental bridge by a better biological substitute namely a lower premolar (Figure 24).

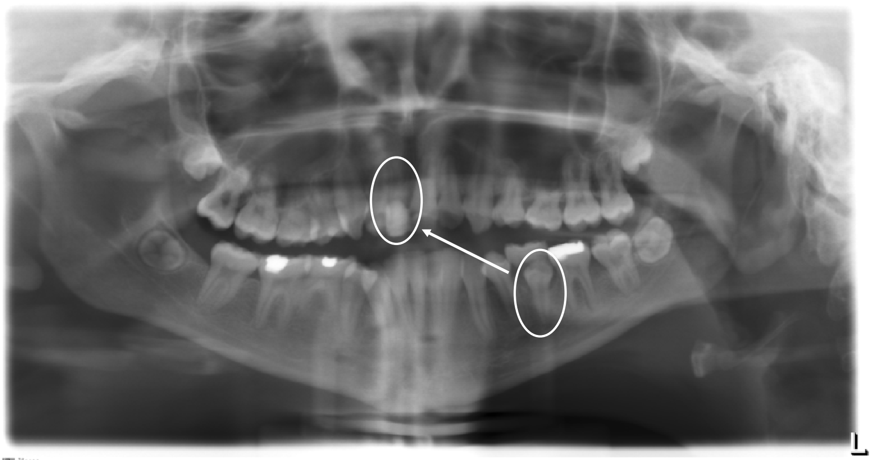

Figure 24: An OPG from a 15-year old girl with a dental bridge at the position of the right upper central incisor and a crowded mandibular arch. Autotransplantation of a mandibular first molar to the position of the dental bridge was proposed as a treatment plan (white circles and arrow).

A cone-beam scan was taken to obtain 3D information for making a template of the donor tooth. In this case the donor tooth is the first premolar in the left mandibula. Cross sections of the cone-beam scan showed that the permanent first molar did not touch with the milk tooth on top of it (Figure 25). This is preferable when making a template because if the teeth did touch, it would be more difficult to construct a template due to similar voxel intensities. Furthermore, the cone-beam scan showed that the donor tooth had an open apex (Figure 25 B).

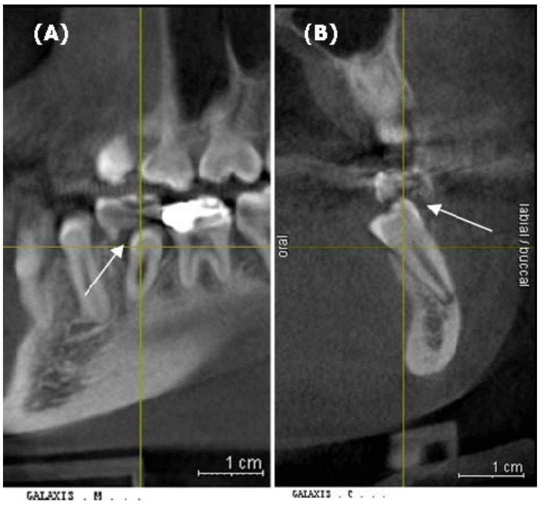

Figure 25: Cross sections of a cone-beam volumetric scan. Both cross sections (A, B) show that the permanent premolar and the milk tooth do not touch (white arrows). The second cross section (B) shows that the donor premolar has an open apex.

Before undertaking the surgery, a donor tooth model was fabricated using computer-aided rapid prototyping (Figure 26). The 3D data obtained from the donor tooth were exported to the ITK-SnAP software for segmentation. Following the results of the prospective study, the segmentation method based on intensity regions was used for tooth segmentation (exact procedure see: Supplemental section 3). After the model was printed out by the STL technique, a handgrip was mounted on the tooth template by a dental technician (Figure 26).

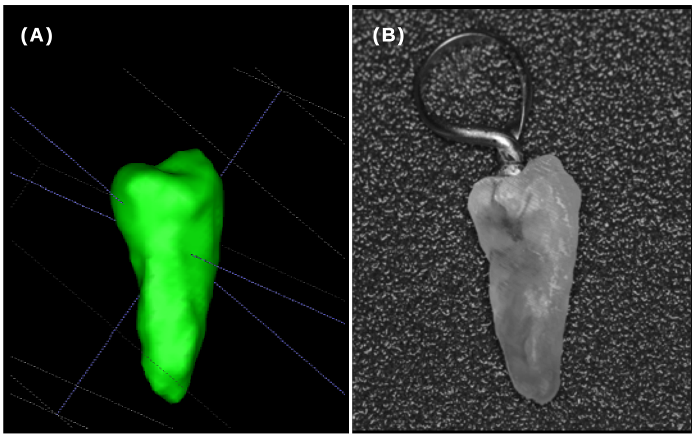

Figure 26: Digital view of the donor tooth after segmentation in ITK-SnAP (A) and the prototyped donor tooth model printed out by the STL technique (B).

Autotransplantation of the premolar took place as a one-stage procedure within 20 minutes. First, the dental bridge at the position of the upper right incisor was removed (Figure 27 A, B and C). Following, the recipient site was prepared using the tooth transplantation template (Figure 27 D). The recipient bone was carefully contoured using a surgical bur until the template fitted well into the recipient site. Next, the donor tooth was carefully extracted (Figure 28) and transferred to the prepared bone socket. The transplanted tooth was placed out of occlusion and fixed with crossed threads (Figure 29).

The use of the prototyped donor tooth model reduced the number of trials with the real donor tooth to fit into the recipient site, thus avoided unnecessary damage to the PDL around the donor tooth.

Besides preservation of the PDL, adaptation between the donor tooth and recipient site is also an important criterion for success after autotransplantation [4, 7]. The recipient socket should be slightly larger than the donor tooth therefore the tooth transplantation template was a little bit larger than the donor tooth (Figure 28). The postoperative examination was not yet carried out at the time of thesis submission.

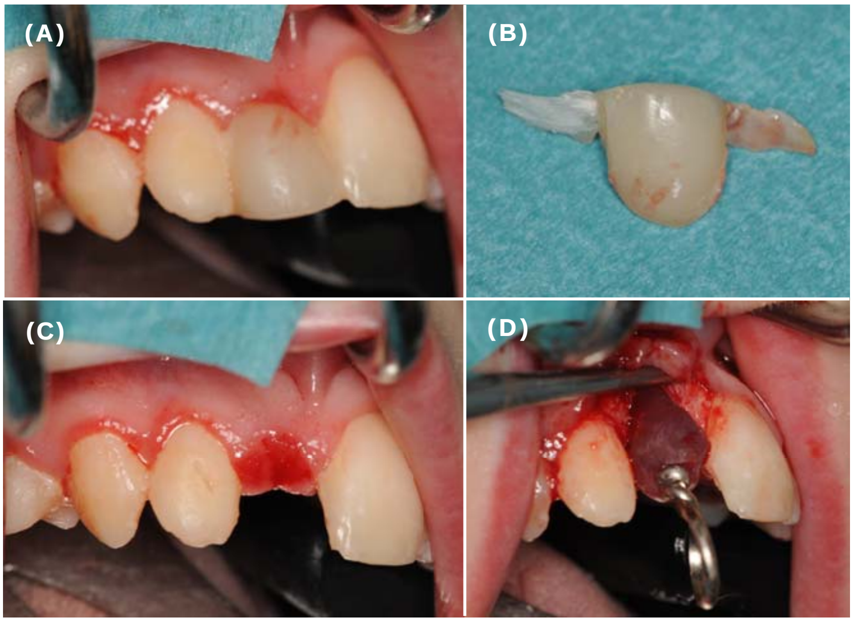

Figure 27: Removal of the dental bridge at the position of the upper right central incisor (A, B, C). Preparation of the recipient socket with the tooth transplantation template (D).

Figure 28: Comparison between the extracted donor tooth and the prototyped tooth model. The donor tooth model is slightly larger than the real donor tooth.

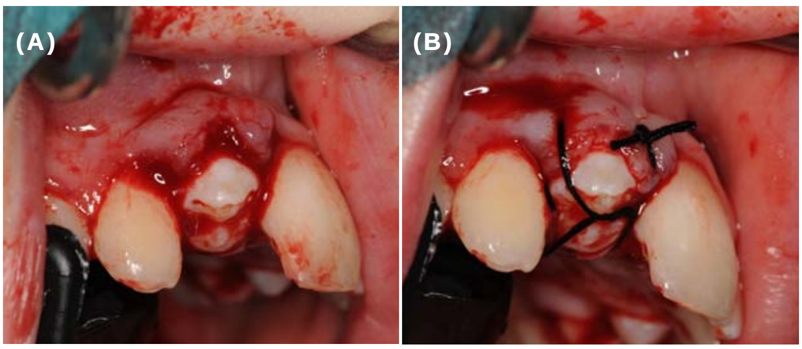

Figure 29: Transplanted first molar without (A) and with fixation in situ (B).

Conclusion

In the present report two different studies were performed to gain more insight in the success of autogenous tooth transplantation. The main advantage of tooth transplantation is that the dentition is preserved using a natural tooth rather than a mechanical prosthesis. When there is a choice between dental implants and transplanted teeth, one should remember that implants are artificial structures and do not have normal periodontal ligaments. Both in clinical sense and from the patient's perspective it makes sense to maintain the use of natural teeth for as long as possible. A prerequisite for the use of autotransplantation is a thorough knowledge of the expected long-term success rate. Patients need to be informed about the long-term outcome of the transplantation because of functional and aesthetic problems when the tooth is lost.

The retrospective study has demonstrated that transplantation of impacted canines may have a successful outcome 11 years after transplantation. Although the success rate was 58.1%, the survival rate was 75.7%. As long as the transplanted canine is present in the mouth, it can be considered as a success because extraction and implant placement has been avoided or at least delayed. Moreover, the age at transplantation was found to be an important prognostic factor confirming the fact that tooth transplantation is preferably done in younger patients. In the future, the results of the retrospective study could be improved by adding more patients and collect pre- and peroperative parameters as complete as possible.

The prospective study indicated region-based segmentation as the most appropriate method for tooth segmentation. Image segmentation is an important step in computer-aided rapid prototyping which can be used for the construction of a tooth transplantation template. A tooth transplantation template may play an important role in the success of autotransplantation. Although it was not confirmed in the present retrospective study, from literature it is known that damage to the periodontal ligament of the donor tooth is a key parameter in the success of tooth transplantation. Computer-aided rapid prototyping makes it possible to construct a template of the donor tooth, which can be used for preparation of the recipient site. Subsequently, the injury of the donor tooth during surgery will be minimized. In this way, using the tooth transplantation template may further increase the success rate of autotransplantation. However, to prove this statement further research is necessary. A clinical study could be performed in which the success rate of teeth transplanted without the use of a template could be compared with the success rate of teeth transplanted with the use of a template.

In the future, it will be important to continue research on success of autogenous tooth transplantation with the intention that autotransplantation of teeth can promise happiness and healthy smiles of patients for a long time.

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Supplemental section 1

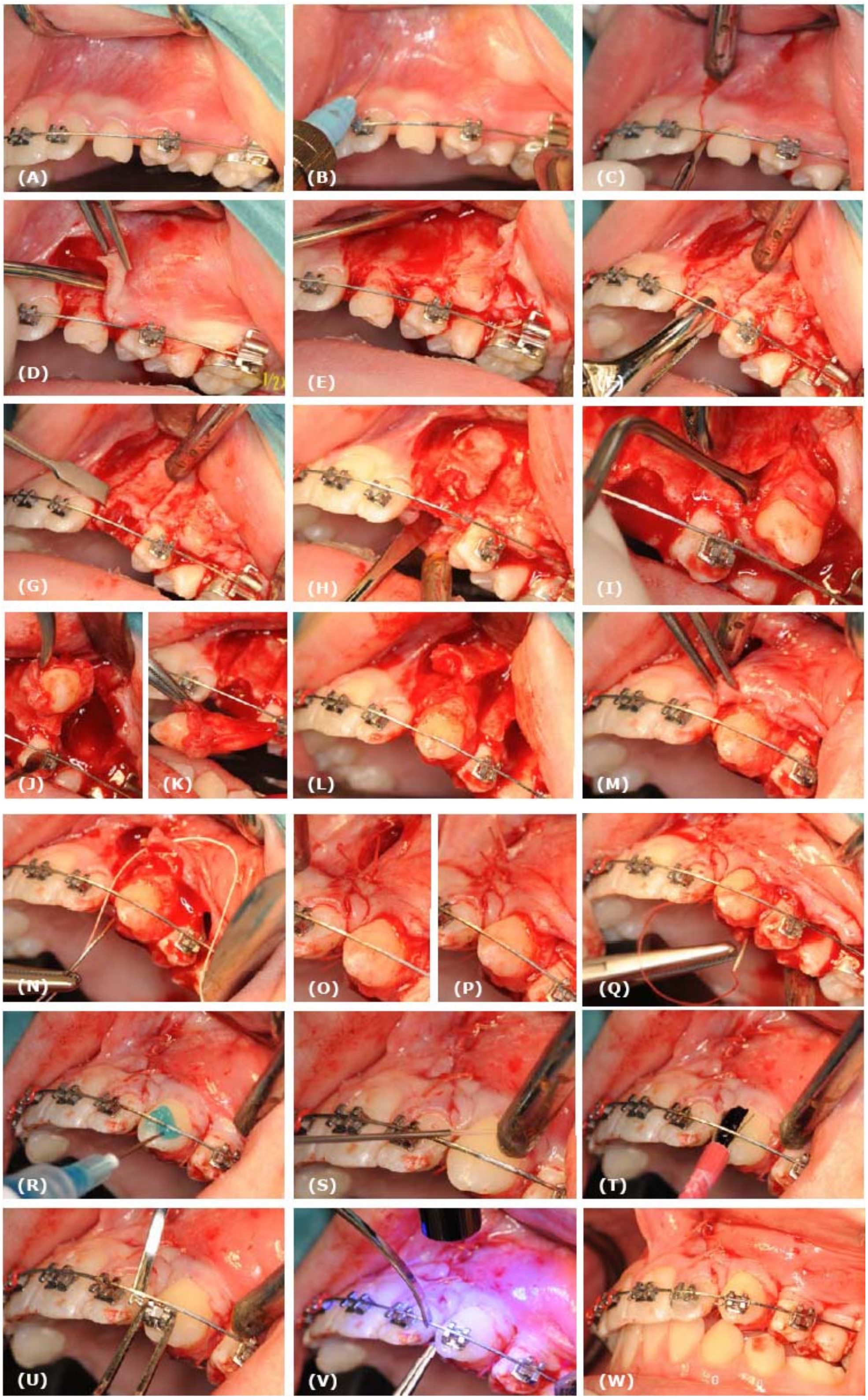

Figure 1: Transplantation of an impacted canine. (A) The canine is labially located, (B) injection of local anaesthesia, (C-E) a trapezoidal incision is made, (F) the remaining primary canine is extracted, (G-H) the recipient socket is prepared and enlarged with chisels, (I-J) removal of the graft, (K) the periodontal ligament is intact, (L) the donor tooth is placed into the recipient socket, (M-Q) the trapezoidal flap is repositioned and sutures are placed, (R-V) the donor tooth is splinted with an transbond XT and orthodontic wire, (W) the occlusion is checked.

Supplemental section 2

Ziekenhuis Oost-Limburg
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Commissie voor Medische Ethie
Registratienummer 056
Erkenningsnummer 371

18-02-2009

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Geachte

Titel studie: SUCCESS OF AUTOGENOUS TOOTH TRANSPLANTATION INFLUENCED BY DIFFERENT PARAMETERS: A RETRO-AND PROSPECTIVE APPROACH

DEFINITIEF GUNSTIG ADVIES

Onze referentie:08/068U
Eudractnummer: B37120095625

Na inzage van de bijkomende informatie en/of aangepaste documenten met betrekking tot bovenvermeld dossier (uw schrijven van 02-02-2009) verleent de Commissie voor Medische Ethiek van het Ziekenhuis Oost-Limburg een gunstig advies voor het eerste deel van deze studie.

Dit gunstig advies van de Commissie houdt niet in dat zij de verantwoordelijkheid voor de geplande studie op zich neemt. U blijft hiervoor dus zelf verantwoordelijk. Bovendien dient u er over te waken dat uw mening als betrokken onderzoeker wordt weergegeven in publicaties, rapporten voor de overheid enz., die het resultaat zijn van dit onderzoek.

U wordt eraan herinnerd dat bij klinische studies iedere door u waargenomen ernstige verwikkeling onmiddellijk zowel aan de opdrachtgever (desgevallend de producent) als aan de Commissie voor Medische Ethiek moet worden gemeld, ook al is het oorzakelijke verband met de studie onduidelijk.

Tenslotte verzoeken wij u ons mee te delen indien een studie niet wordt aangevat, of wanneer ze wordt afgesloten of vroegtijdig onderbroken (met opgave van eventuele reden).

Met de meeste hoogachting

Prof. Dr. E. de Jonge
voorzitter Commissie voor Medische Ethiek

SECRETARIAAT Commissie voor Medische Ethiek ZOL
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Supplemental section 3

First, a three-dimensional scan is taken using the cone-beam scanner Galileos to indicate the tooth that will be used as a transplant. Following, the DICOM data are exported from the Galileos software to the segmentation program ITK-SnAP. A key feature of ITK-SnAP is the ability to segment and navigate through the volumetric data set in any of the orthogonal slice windows (sagittal, coronal and axial views).

The segmentation procedure starts with selecting the region of interest; this is a sub-region of the image on which to perform the segmentation (Figure 1). By doing this, the amount of memory and the time required to complete segmentation can be reduced.

After adjusting the region of interest, the 'Segment 3D' button can be pressed to start segmentation (Figure 2). In the **first step** of preprocessing the option 'intensity region' is selected because this is the most suitable method for tooth segmentation (see prospective study: results and discussion). When the 'preprocess image' button is pressed a window appears to specify the intensity region filter. The goal in setting the parameters is to make sure that the voxels inside the tooth are assigned positive values and the voxels outside the tooth are assigned negative values. In other words, the intensity range of the tooth has to be estimated. This can be done by moving the cursor in and around the tooth and simultaneously looking at the values of the grey level intensity, reported in the box 'Grey' (red circle in Figure 2). In this particular case the intensities in the tooth range between 648.6 (lower threshold) and 2724.9 (upper threshold). All voxels which have intensity lower or higher than these values do not belong to the tooth. The smoothness value has an effect on the smoothness of the region growing. The resulting image after determining the intensity range of the tooth is shown in Figure 3.

The **second step** of preprocessing is used to position spherical bubbles that initialize the region growing. Several bubbles are randomly placed in the tooth by navigating through all slices (Figure 4). The radius slider can be used to change the radius of the bubbles. In the **third step** automatic segmentation can be started, the region starts growing from the bubbles which were placed in the tooth (Figure 5). After finishing the automatic segmentation, some tooth boundaries may not be completely segmented (Figure 6). Therefore, the segmented image can be further manual checked using the paintbrush tool (Figure 7).

After manual editing is completed the segmentation result is saved as STL files. Following, the STL file is sent to SKM Rapid Modelling B.V. (Helmond, Netherlands). The STL file is exported to the stereolithography machine for fabrication and then the printed template is sent back to the department OMF of the St. John's Hospital (Genk, Belgium). Finally, the template is submitted to a quality control and further modified (construction of a handgrip) by a technician in the digital dental laboratory of Aporis nv (Diepenbeek, Belgium).

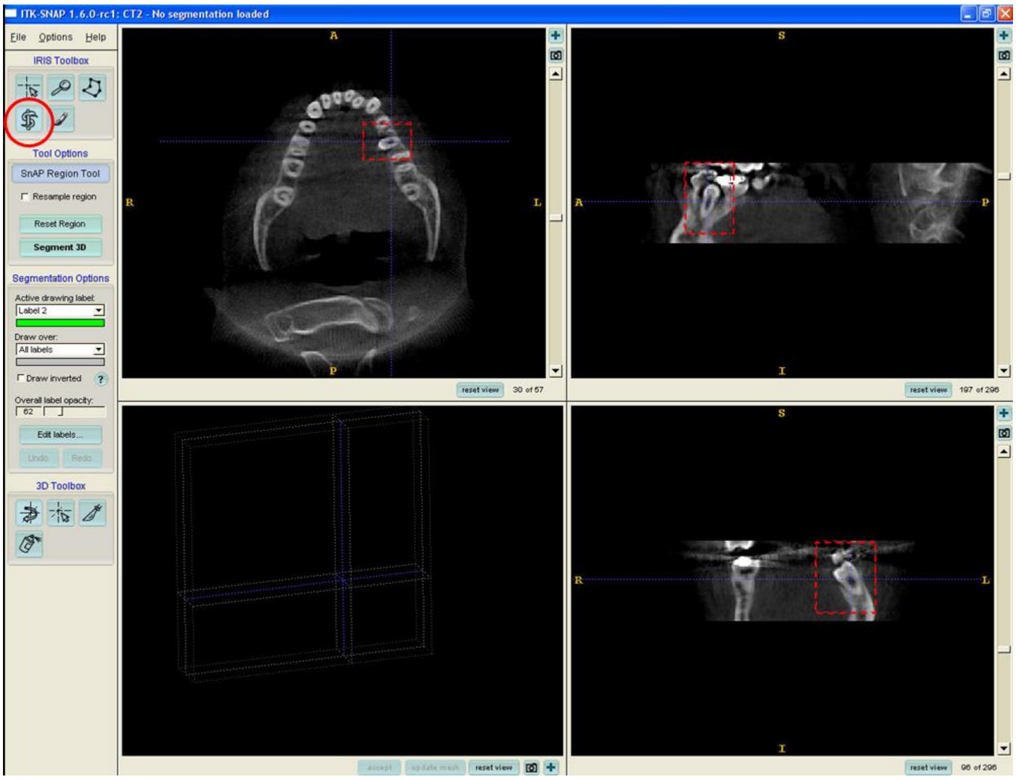

Figure 1: The red dashed selection box displays the tooth that will be segmented. To select the region of interest the snake tool (red circle) can be used.

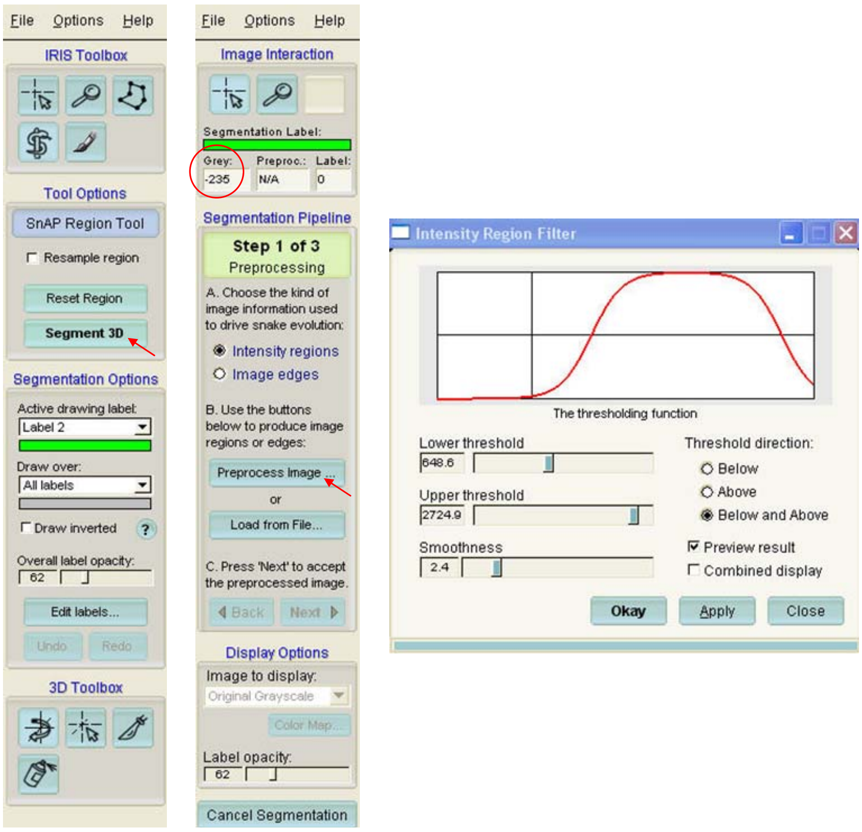

Figure 2: To start segmentation the 'Segment 3D' button is pressed (left red arrow). The first step of segmentation is preprocessing the image (right red arrow), which means that the intensity range of the tooth has to be estimated. This can be done by moving the cursor around the tooth and simultaneously looking at the values of the grey level intensity (red circle). In the intensity region filter window the lower and upper threshold can be adjusted.

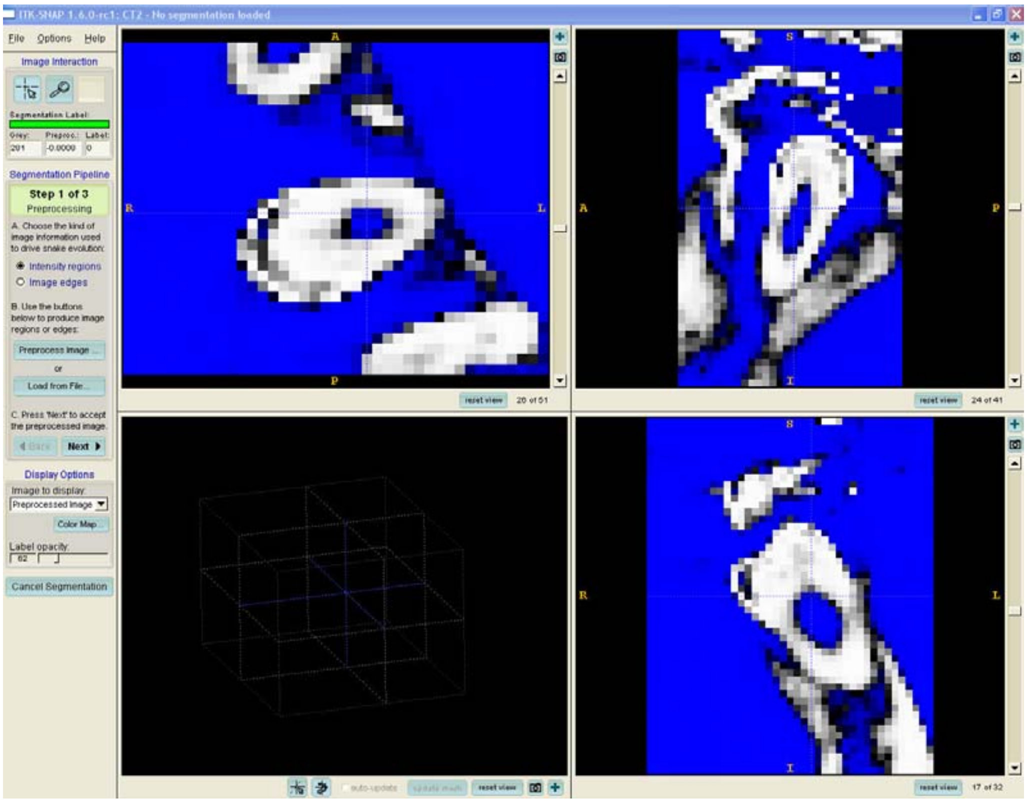

Figure 3: The resulting image after selecting the region of interest and the intensity range of the tooth.

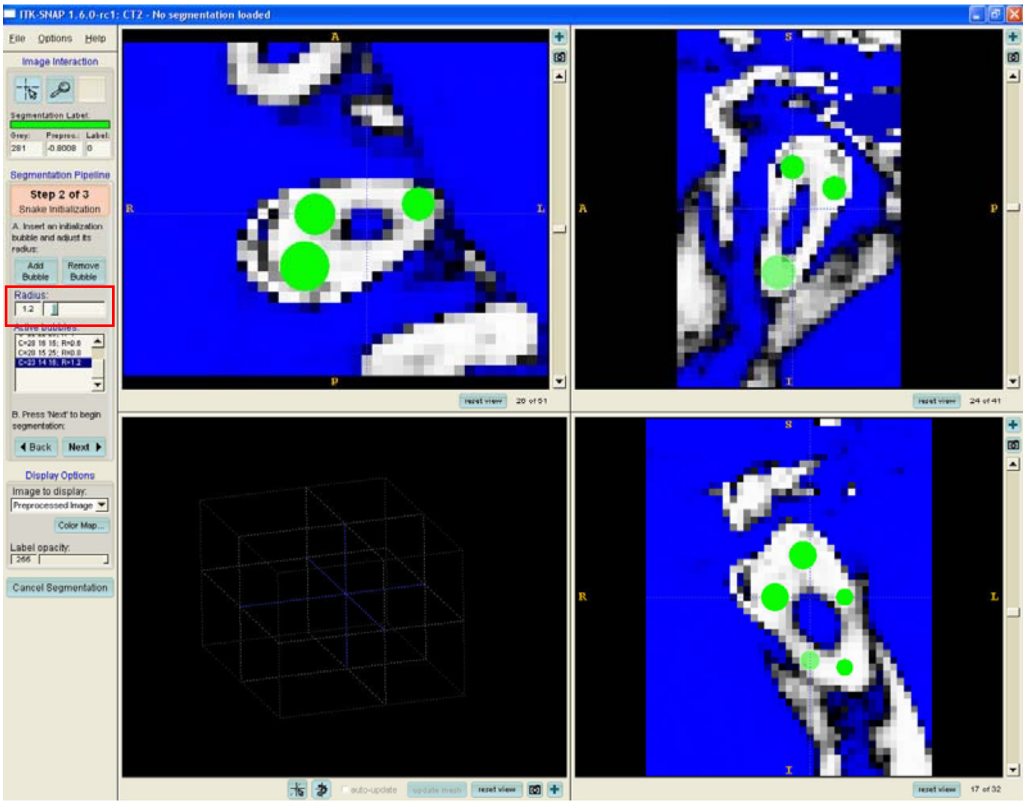

Figure 4: Spherical bubbles (green) are randomly placed in the tooth by navigating through all slices. The red box indicates the radius slider to changes the radius of the bubbles.

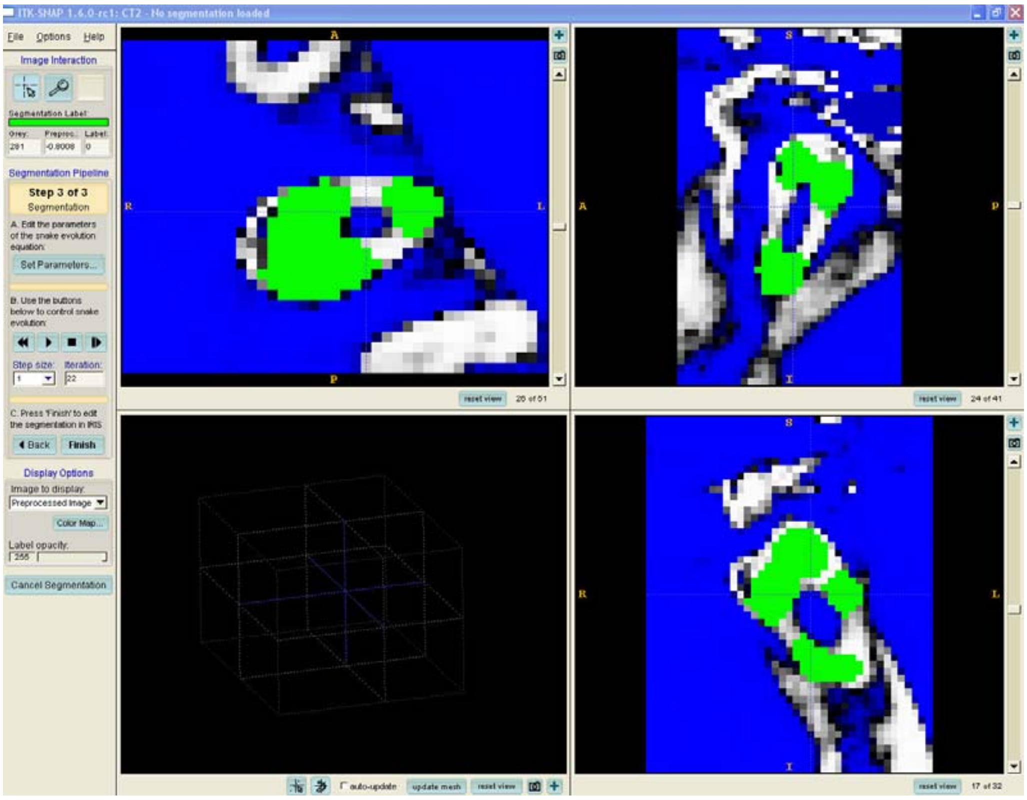

Figure 5: The region starts to grow from the bubbles which were placed in the tooth.

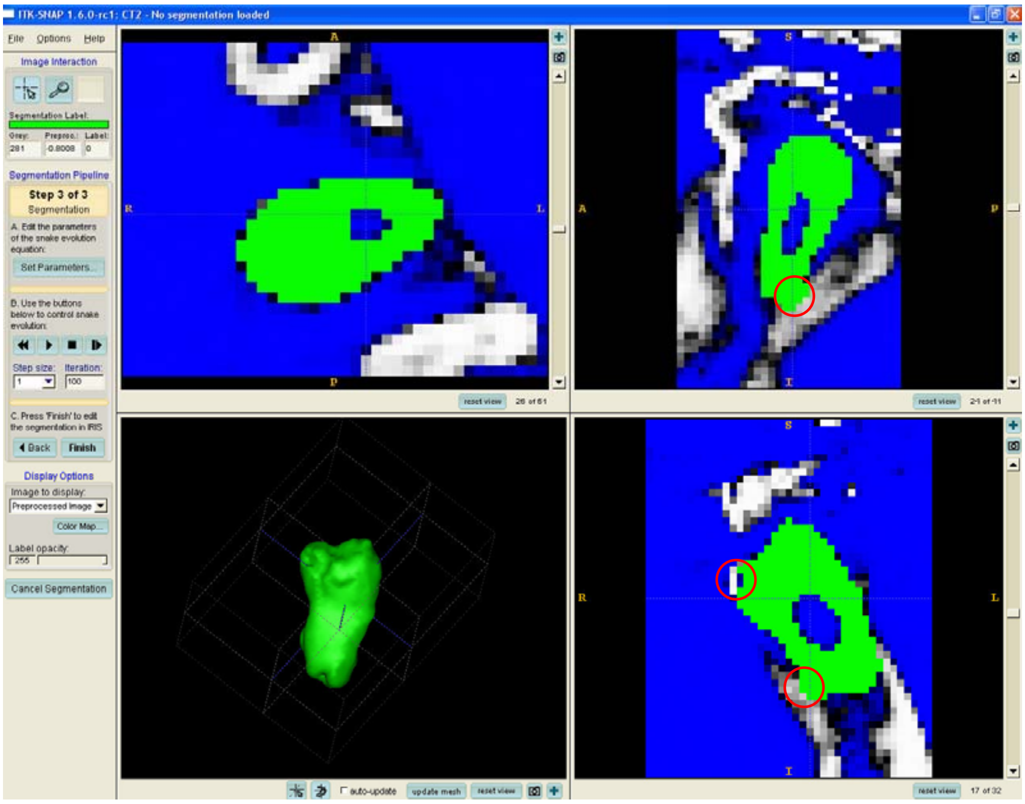

Figure 6: When automatic segmentation is stopped, some tooth boundaries are not completely segmented (red circles).

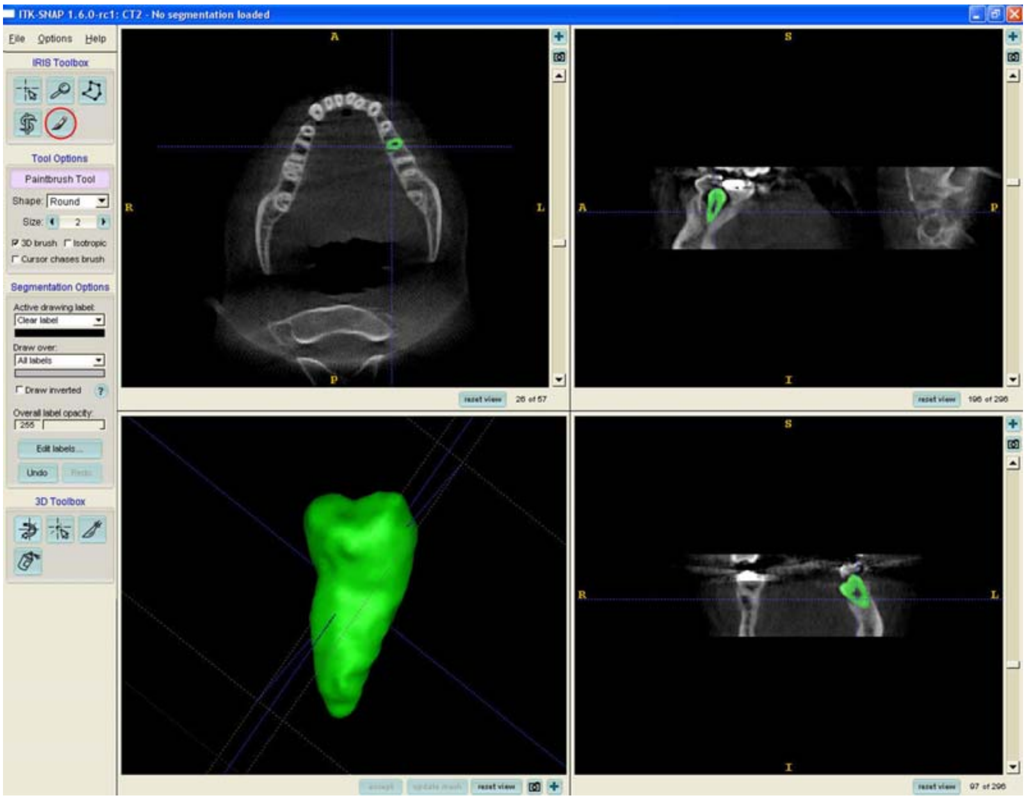

Figure 7: When the automatic segmentation is finished, the paintbrush tool (red circle) can be used for manual checking of the tooth boundaries.

Supplemental section 4

3D SYSTEMS

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-- Scott Turner - President - Scicon Technologies

TECHNICAL DATA

Liquid Material

MEASUREMENT	CONDITION	VALUE:
Appearance		Clear
Liquid Density	@ 25 °C (77 °F)	1.13 g/cm³
Solid Density	@ 25 °C (77 °F)	1.21 g/cm³
Viscosity	@ 30 °C (86 °F)	150 - 180 cps
Penetration Depth (Dp)*		6.3 mils
Critical Exposure (Ec)*		7.6 mJ/cm²
Tested Build Styles		EXACT™, FAST™, QuickCast™

Post-Cured Material

MEASUREMENT	CONDITION	VALUE:
Tensile Strength	ASTM D 638	58 - 68 MPa (8410 - 9860 PSI)
Tensile Modulus	ASTM D 638	2690 - 3100 MPa (390 - 450 KSI)
Elongation at Break (%)	ASTM D 638	5 - 13 %
Flexural Strength	ASTM D 790	87 - 101 MPa (12620 - 14650 PSI)
Flexural Modulus	ASTM D 790	2700 - 3000 MPa (392 - 435 KSI)
Impact Strength (Notched Izod)	ASTM D 256	15 - 25 J/m (0.3 - 0.5 ft- lb/in)
Heat Deflection Temperature	ASTM D 648 @ 66 PSI @ 264 PSI	 53 - 55 °C (127 - 131 °F) 48 - 50 °C (118 - 122 °F)
Hardness, Shore D		86
Co-efficient of Thermal Expansion	ASTM E 831-93 TMA (T<Tg, 0 - 40 °C) TMA (T<Tg, 75 - 140 °C)	 71 µm/m-°C 153 µm/m-°C
Glass Transition (Tg)	DMA, E"	58 °C (136 °F)

* Dp/Ec values are the same on all systems.

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PN 70712 Issue Date - 26 Apr 06

Supplemental section 5

Table 1: Values of the surface distance (mm) for the 10 molar teeth.

Surface distance (mm)		
Molar teeth	**Intensity-based**	**Edge-based**
1	0.30	0.33
2	0.19	0.15
3	0.31	0.30
4	0.29	0.21
5	0.30	0.19
6	0.24	0.26
7	0.25	0.28
8	0.21	0.23
9	0.19	0.25
10	0.30	0.29
Mean ± SD	**0.26 ± 0.05**	**0.25 ± 0.06**

Table 2: Values of the segmentation time (minutes) for the 10 molar teeth.

Segmentation time (minutes)		
Molar teeth	**Intensity-based**	**Edge-based**
1	30	45
2	23	33
3	12	33
4	8	27
5	10	19
6	17	25
7	15	20
8	10	20
9	10	22
10	10	20
Mean ± SD	**15 ± 7**	**26 ± 8**